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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/54, C07K 14/71, 16/28, A61K 38/17, 39/395

A1

(11) International Publication Number: WO 97/23629

(43) International Publication Date: 3 July 1997 (03.07.97)

(21) International Application Number: PCT/AU96/00826

(22) International Filing Date: 20 December 1996 (20.12.96)

(30) Priority Data:
PN 7277 22 December 1995 (22.12.95) AU

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: A NOVEL RECEPTOR-TYPE TYROSINE KINASE AND USE THEREOF

(57) Abstract

The present invention relates generally to a novel receptor-type tyrosine kinase, to genetic sequences encoding same and to uses therefor. More particularly, the present invention contemplates a receptor-type tyrosine kinase having the following properties: (i) belongs to the Eph subfamily of RTKs as determined by conserved cysteine residues and fibronectin type III repeats; (ii) comprises protein tyrosine kinase catalytic domain motifs; and (iii) comprises an amino acid sequence substantially as set forth in SEQ ID NO: 2 or having at least about 79 % similarity thereto.

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A NOVEL RECEPTOR-TYPE TYROSINE KINASE AND USE THEREOF

The present invention relates generally to a novel receptor-type tyrosine kinase, to genetic sequences encoding same and to uses therefor.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

The receptor tyrosine kinases (RTKs) form an important class of molecules involved in the regulation of growth and differentiation of cells.

The RTKs are transmembrane molecules which transduce signals from the extracellular environment into the cytoplasm. They include well-studied regulators of cell proliferation and differentiation such as *c-kit* and the receptors for epidermal growth factor, platelet-derived growth factor and macrophage colony-stimulating factor (1). Signalling is initiated when a cognate ligand binds to the RTK extracellular domain. This triggers a sequence of events resulting in the activation of an intracellular tyrosine kinase domain. Critical to this process is ligand-mediated receptor dimerization and reciprocal tyrosine phosphorylation by the dimerized molecules (2). Once their catalytic domain is activated, RTKs can bind and phosphorylate specific intracellular proteins which act as second messengers.

Eph was the first-isolated member of a new subfamily of RTKs (3). This group is distinguished by the sequential arrangement of a cysteine-rich region and two fibronectin-type-III repeats in the extracellular domain (4). At least 28 members have now been identified making this the largest subfamily of RTKs. They have been found in diverse species, including zebrafish (5), frogs (6), chickens (4,7,8), mice (7,9,10-12,13), rats (14,15) and humans (3,16,17,18,19). Certain features of the expression pattern of the Eph subfamily suggest key functions during embryonic development. First, strong expression in the embryo is characteristic (4,7,8,9,11,20). Expression is generally down-regulated later in development, but often continues in the adult at a restricted number of tissue sites. Secondly, *in situ* hybridization and immunolocalization studies have identified associations between the expression of specific Eph-subfamily molecules and particular events in morphogenesis. For example, Eck is transiently expressed in cells adjacent to the primitive streak during gastrulation and later its transcripts are found in specific rhombomeres of the developing hindbrain and in the ectoderm of the second and third branchial arches (21).

The expression of Nuk protein on growing peripheral nervous system axons, which disappears when the axons have ceased migrating, and the segment-restricted pattern of Nuk and Sek expression during hindbrain morphogenesis, are other examples (9,11). Preferential expression at interfaces between embryonic cell populations and in intercellular junctions has led to the suggestion that Eph-subfamily molecules influence embryonic differentiation and cellular migration by interactions involving direct cell-cell contact (11,21). The recent finding that ligands for some members of this group are cell membrane bound supports this notion (22-25). Eph-subfamily kinases are also significant because of a potential role in oncogenesis. Eph and Erk are over expressed in some epithelial tumor cell lines and carcinomas (3,26), while Hek overexpression occurs sporadically in leukemia (32). Furthermore, artificial overexpression of Hek or Eph in NIH-3T3 cells resulted in a transformed phenotype, as evidenced by the ability to form colonies in agar and tumors in nude mice (27). These molecules may also be involved in tumor progression. In transgenic models of murine mammary cancer, overexpression of the Eph-subfamily members Myk-1 and Myk-2 correlated with the development of poorly-differentiated and invasive tumors (13).

Embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst (28). They are undifferentiated and totipotent. When grown *in vitro* in the absence of leukemia inhibitory factor (LIF), they develop into embryoid bodies containing cells committed to a variety of tissue lineages (29). This system provides a convenient model of very early embryonic development, which is difficult to study *in vivo* because of problems in harvesting preimplantation embryos and the small amounts of tissue involved.

In work leading up to the present invention, the inventors considered that RTKs expressed by ES cells and embryoid bodies are likely to be involved in the initial differentiation and organization of embryonic tissues. To analyse this further, the inventors used reverse transcriptase (RT) -mediated polymerase chain reaction (PCR) [RT-PCR] to identify Eph subfamily RTKs in ES cells. In accordance with the present invention, the inventors have identified a novel RTK member from the Eph-subfamily.

Accordingly, one aspect of the present invention provides a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a novel member of the Eph subfamily of RTKs or a derivative, homologue or chemical analogue thereof.

More particularly, the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an RTK or a derivative, homologue or chemical analogue thereof having the following characteristics:

(i) belongs to the Eph subfamily of RTKs as determined by conserved cysteine residues and fibronectin type III repeats;

(ii) comprises protein tyrosine kinase catalytic domain motifs; and

(iii) comprises an amino acid sequence substantially as set forth in Figure 2 [SEQ ID NO:2] or having at least about 79% similarity to all or part thereof.

Preferably, the novel RTK of the present invention is of animal or mammalian origin. Preferred mammals include but are not limited to humans, primates, livestock animals (e.g. sheep, cows, horses, pigs, donkeys), laboratory test animals (e.g. rabbits, mice, rats, guinea pigs), companion animals (e.g. dogs, cats) or captive wild animals (e.g. foxes, kangaroos, deer). Preferred non-mammalian animals include fish and birds. The present invention is particularly exemplified herein by reference to a novel RTK of murine origin but this is done with the understanding that the present invention extends to all animal and mammalian homologues of the novel murine Eph-subfamily RTK and in particular a human form of the RTK. Hereinafter, the novel RTK of the present invention is referred to as "Esk" for "embryonic stem cell kinase".

The present invention extends to derivatives, homologues and chemical analogues of Esk, which Esk has an amino acid sequence set forth in Figure 2 [SEQ ID NO:2]. Derivatives include single or multiple amino acid substitutions, deletions and/or additions to the sequence and encompass mutants, part and fragments thereof. The term "derivative" also encompasses soluble or solubilized or otherwise secreted forms of the Esk molecule. Derivatives also encompass chimeric molecules comprising Esk or a derivative, homologue, or analogue thereof and at least one other molecule such as another receptor or ligand.

Homologues include novel Esks of animal or mammalian origin having at least about 79%, more preferably at least about 85%, even more preferably at least about 90% and still more preferably at least about 95% or above sequence similarity to the amino acid sequence set forth in Figure 2 [SEQ ID NO:2]. Preferred homology comparisons are done between coding regions or 3′ or 5′ regulatory regions or particularly conserved regions.

Analogues of Esk contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methOds which impose conformational constraints on the peptides or their analogues. Such analogues may also provide stability to molecule administered *in vivo* or for manipulation of molecules *in vitro*.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5'-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of the incorporation of unnatural amino acids and derivatives during polypeptide

synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids contemplated for use in accordance with the present invention is given in Table 1.

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
carboxylate		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-	Norb	L-N-methylglutamine	Nmgln
carboxylate		L-N-methylglutamic acid	Nmglu
cyclohexylalanine		Chexa L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug

D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva
D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr

D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp
D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
L-ethylglycine	Etg	penicillamine	Pen
L-homophenylalanine	Hphe	L-α-methylalanine	Mala
L-α-methylarginine	Marg	L-α-methylasparagine	Masn
L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
L-α-methylserine	Mser	L-α-methylthreonine	Mthr
L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr

L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

Particularly useful derivatives contemplated by the present invention are soluble forms of the Esk receptor. A soluble receptor is also referred to herein as a secreted Esk protein and is most preferably in recombinant form. Soluble Esk molecules are useful reagents for ligand isolation, as antagonists of Esk-ligand interaction, as a substrate for antibody production which antibodies are in turn useful diagnostic reagents.

Accordingly, another aspect of the present invention provides a secreted recombinant Esk protein or derivative thereof comprising conserved cysteine residues and fibronectin type III repeats and a portion of the amino acid sequence substantially set forth in SEQ ID NO:2 or having at least 79% homology thereto which is not part of the membrane bound region of the corresponding anchored receptor. Preferably, the amino acid sequence of the secreted

recombinant Esk is encoded by a nucleotide sequence substantially as set forth in SEQ ID NO:2 or having at least 82% homology thereto.

In a particularly preferred embodiment, the nucleic acid molecule comprises a nucleotide sequence (or a complementary form thereof) substantially as set forth in Figure 2 [SEQ ID NO:1] or having at least about 82% similarity to all or part thereof or is capable of hybridising to the sequence set forth in SEQ ID NO:1 or a complementary form thereof under low stringency conditions.

Preferred percentage nucleotide similarities include at least about 84%, more preferably at least about 90% and even more preferably at least about 95% or above. The nucleic acid molecules of the present invention include single or multiple nucleotide substitutions, deletions and/or additions to the nucleotide sequence set forth in Figure 2 [SEQ ID NO:1] and mutants, parts and fragments thereof, which are all encompassed by the term "derivative" of the nucleotide sequence set forth in Figure 2 [SEQ ID NO:2].

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

The nucleic acid molecules of the present invention are preferably carried by a vector molecule and more particularly an expression vector. Preferred expression vectors direct expression in mammalian, insect and/or bacterial cells. The present invention also extends to

cells carrying the recombinant nucleic acid molecules of the present invention.

Preferred nucleic acid molecules are DNA and more preferably cDNA.

The present invention is also directed to a recombinant Esk polypeptide having the following characteristics:

(i) is an RTK belonging to the Eph subfamily of RTKs as determined by cysteine residues and fibronectin type II repeats;

(ii) comprises protein tyrosine kinase catalytic domain motifs; and

(iii) comprises an amino acid sequence substantially as set forth in Figure 2 [SEQ ID NO:2] or having at least about 79% similarity to all or part thereof.

The recombinant Esk is preferably in isolated form meaning that a composition comprises at least about 20%, more preferably at least about 30%, still more preferably at least about 40-50%, even more preferably at least about 60-70% and yet even more preferably at least about 80-90% or above of Esk as determined by activity, molecular weight, or immunological reactivity. The preparation may also be sequencably pure or of a purity suitable for use in a pharmaceutical composition.

The present invention extends to the ligand(s) of Esk and to agonists and antagonists of Esk-ligand interaction. Agonists and antagonists may be, for example, antibodies or derivatives of the Esk or derivatives of the ligand. Derivatives of Esk or its ligand include soluble or solubilised forms thereof. Reference herein to "Esk" includes both anchored forms (ie. membrane bound forms) of the receptor as well as soluble (ie. secreted) forms of the receptor.

Modulating expression of Esk may have important potential in therapeutic regimens for the treatment or prophylaxis of cancers caused or exacerbated by aberations in Esk or aberations in Esk-ligand interaction. This will be particularly important for the treatment of mucositis. This condition remains the major adverse effect of chemotherapy and radiotherapy of malignant disease. As Esk is expressed in all epithelial tissues, modulation of Esk-ligand

interactions may have therapeutic applications for skin (such as following burns, abrasions, eczema and other forms of skin wounding leading to skin loss or damage); hair (hair loss or abnormal hair growth); corneal ulcers (injury, infection, Sjogrens syndrome and related autoimmune diseases; mucositis (mouth, oesophagus, stomach, small and large bowel); infective ulcerative colitis and other non-infective inflammatory enteritides; peptide ulcers; oesophageal reflux; Sjogrens syndrome and related autoimmune diseases; infection of other mucous surfaces (eg. vaginitis and vulvitis); Sjogrens syndrome and related autoimmune diseases; infection of the lung (eg. shock lung, inhalation of noxious fumes, infection); liver (eg. regeneration after viral illness or toxic damage); pancreatitis; urological disease involving tubules, pelvicalyceal system, ureters, bladder or urethra and salivary glands.

Accordingly, the present invention contemplates a method for modulating Esk-ligand interaction in an animal, said method comprising administering to said animal a modulating effective amount of an agonist or antagonist of Esk-ligand interaction. The term "modulating" includes facilitating Esk-ligand interaction or inhibiting, reducing or otherwise interfering with Esk-ligand interaction. Either form of modulation may be required depending on, for example, the type of treatment such as the treatment of cancer or the promotion or inhibition of cell apoptosis.

The present invention, therefore, contemplates a pharmaceutical composition comprising an Esk-ligand interaction modulating effective amount of an agonist or antagonist of Esk-ligand interaction and one or more pharmaceutically acceptable carriers and/or diluents.

The formation of pharmaceutical compositions is generally known in the art and reference can conveniently be made to Remington's Pharmaceutical Sciences, 17th end., Mack Publishing Co., Easton, Pennsylvania, USA.

The active ingredients of a pharmaceutical composition comprising the Esk agonists or antagonists or their derivatives are contemplated herein to exhibit excellent therapeutic activity, for example, in modulating Esk-ligand interaction when administered to an animal

in an amount which depends on the particular case. For example, from about 0.5 μg to about 20 mg per kilogram of body weight per day may be administered. Dosage regimes may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly or monthly, or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. The active compounds may be administered in any convenient manner such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes or by implanting (eg using slow release molecules), topical administration or following or during surgery or biopsy or other invasive procedure. Depending on the route of administration, the active ingredients which comprise the Esk agonists or antagonists or chemical analogues thereof may be required to be coated in a material to protect said ingredients from the action of enzymes, acids and other natural conditions which may inactivate said ingredients. In order to administer Esk agonists or antagonists by other than parenteral administration, they will be coated by, or administered with, a material to prevent its inactivation. For example, homologues may be administered in an adjuvant, co-administered with enzyme inhibitors or in liposomes. Adjuvants contemplated by the present invention include, but are not limited to, cytokines (e.g. interferons) as well as resorcinols, non-ionic surfactants such as polyoxyethelene oleyl ether and n-hexadecyl polyethylene ether.

The active compounds may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for

example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solven with various of the ingredients enumerated above, as required, followed by filtered sterilisation. Generally, dispersions are prepared by incorporating the various sterilised active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation of vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the Esk agonists or antagonists or chemical analogues thereof are sutiably protected as described above, the active, compound may be orally administered, for example, with an inert diluent or with a assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tables, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between 5 to about 80% of

the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that a oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

A pharmaceutically acceptable carrier and/or diluent inclues any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the acive ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

Its is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mamallian subjects to be treated;

each unit coating a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic affect to be achieved, and (b) the limiations inherent in the art of compound such a active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principial active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 μg to about 2000 mg/ml of carrier. In the case of compositions coating supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The animal to be treated is preferably a mammal such as a human, primate, livestock animal, laboratory test animal, companion animal or captive wild animal. Most preferably, the animal is human.

The present invention further extends to antibodies to the Esk molecules herein described. The antibodies may be monoclonal or polyclonal. Antibodies to the Esk molecules of the present invention are useful as therapeutic agents in modulating Esk-ligand interaction or as diagnostic agents to assay for Esk molecules or Esk-ligand internation. Assay techniques are well known in the art and include, for example, sandwhich assays and ELISA.

Accordingly, another aspect of the present invention contemplates a method for assaying for Esk expression on a cell, said method comprising contacting a biological sample containing cells putatively expressing Esk with an Esk-binding effective amount of an antibody thereto

for a time and under conditions sufficient for said antibody to bind to said Esk and then detecting said Esk-antibody binding.

The presence of Esk on a cell can be detected using a wide range of immunoassay techniques such as those described in US Patent Nos. 4,016,043, 4,424,279 and 4,018,653. This includes both single-site and two-site, or "sandwhich", assays of the non-competitive types, as well as in the traditional competitive binding assays. Sandwhich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwhich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, and Esk antibody is immobilised onto a solid substrate to form a first complex and the sample containing cells to be tested brough into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an Esk-antibody secondary complex, an antibody, labelled with a receptor molecule capable of producing a detectable signal and specific to another antigen to the cell, is then added and incubated, allowing time sufficient for the formation of a tertiary complex of Esk-antibody-labelled antibody. Any unreacted material is washed away, and the presence of the first antibody is determined by observation of a signal produced by the reporter molecule on the second antibody. The results may either be qualitative, by simple observation of the visible signal or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations of the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody, or a reverse assay in which the labelled antibody and sample to be tested are first combined, incubated and then added simultaneously to the bound antibody.

These techniques are well known to those skilled in the art, and the possibility of minor variations will be readily apparent. Alternatively, a labelled Esk antibody may be added directly to the sample of cells and the reporter molecule defected.

The solid substrate is typically glass or a polymer, the most commonly used polymers being

cellulose, polyacrylamide, nylon, polystyrene polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing the molecule to the insoluble carrier.

By "reporter molecule", as used in the present specification, is meant a molecule which, by its chemical nature, profices an analytical identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecule in this type of assay re either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes). In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognised, however, a wide variety of different conjugation techniques exist which are readily available to one skilled in the art. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. It is also possible to employ fluorogenic substrates which yield a fluorescent product.

Alternatively, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state of excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining ternary complex is then exposed to the light of the appropriate wavelength, the fluorescent observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are particularly preferred for the present method. However, other reporter molecules, such

as radiosotope, chemiluminescent or bioluminescent molecules, may also be employed. It will be readily apparent to the filled technician how to vary the procedure to suite the required purpose.

The present invention further extends to genetic molecules derived from an Esk gene useful as probes, antisense or sense molecules for diagnostic or therapeutic situations.

The present invention is further described by the following non-limiting Figures and/or Examples.

Reference herein to "similarity" in relation to amino acid or nucleotide acid or nucleotide sequences has substantially the same meaning as "homology" and "identify".

In the Figures:

Figure 1 is a diagrammatic representation showing the position of the four degenerate oligonucleotide primers (PI-P4) used for RT-PCR. The relatively-conserved peptide motifs on which the primers were based are shown above a schematic representation of the basic domain structure of Eph-subfamily molecules. The particular amino acid sequences used in the figure are from eph. P1 and P2 were sense primers: P3 and P4 were antisense primers. Their sequences were as follows:

P1, 5′-GTAGGCATGCAAGGAGAC(AC)TT(CT)AACC-3′ [SEQ ID NO:3];
P2, 5′-GCGATGATCAT(CG)AC(AGT)GA(AG)TA(CT)ATGG-3′ [SEQ ID NO:4];
P3, 5′-GTAGGAATTCCA(CGT)ACATC(AG)CT(AG)GC-3′ [SEQ ID NO:5];
P4, 5′-CCA(TA)A(AG)CTCCA(CT)ACATC(AG)CT-3′ [SEQ ID NO:6].

TMD denotes the transmembrane domain.

Figure 2 is a reprsentation showing the nucleotide and deduced amino acid sequence of Esk. The signal peptide and transmembrance domain are boxed. Conserved cysteine residues in the extracellular domain are circled and two fibronectin type III repeats are in a striped box. In the catalytic domain, arrowheads indicate the highly-conserved Gly-X-Gly-X-X-Gly motif and a dot marks the invariant lysine residue. Two motifs assocaited with substrate specificity for tyrosine are underlined. The stop codon which terminates the coding region is indicated by an asterisk.

Figure 3 is a photographic representation of expression of Esk. Northern blots of poly(A)$^+$ RNA from day-12 mouse embryo and the adult mouse tissues shown were hybridized to a ^{32}P-labelled probe derived from clone 35C15. This was then stripped from the filters and hybridization to a GAPDH probe was performed. The positions of RNA size markers are indicated to the left of the blots.

Figure 4 is a photographic representation showing Northern blot of ES cell, embyoid body and embryonic firbroblast poly (A)$^+$ RNA hybridized to ^{32}P-labelled probes synthesized from Esk,, Mek4 and Eck cDNA. Probes were stipped from the filter between reprobingS. Hybridisaztion to a GAPDH probe was [erformed last. The sizes of transceipts are indicated to the right of the figure.

Figure 5 is a diagrammatic representation of all Eph-subfamily clones isolated from ES cells using RT-PCR aligned with the full-length cDNA clone derived from a λZAP library. Numebrs represent nucleotides in the Esk full length sequence. ECD, extracellular domain; ICD, intracellular domain.

Figure 6 is a reprsentation showing a plot of grains after scoring approximately 130 Chromosomes 6, showing probable localisation of Esk to bands B1-B2. Grains scored from C57BL and BALB/c mice are represented with solid and open dots respectively.

Figure 7 is a photographic representation of an analysis of the relationship between Esk and

Eph by Southern hybridization. A mouse genomic Southern blot was prepared using DNA digested with the restriction enzymes shown. This was initially hybridized in 40% formamide to a probe derived from the catalytic domain of human Eph (A). The membrance was then stripped and rehybridized in 50% formamide to a prpbe derived from equivalent sequence in mouse Esk(B). The position of DNA size markers is indicated at left.

Figure 9 is a photographic representation showing *in situ* analysis of Esk expression in whole-mounted embryoid bodies (A) and day 9.5 mouse embryos (B). Embryoid bodies were differentiated *in vitro* from ES cells by culturing without feeder cells in LIF-deficient mediu, for 7-10 days. Sense and antisense digoxigenin-labelled riboprobes were synthesized from cDNA fragments of Esk and hybridized to the whole-mounts. Bound probe was detected using alkaline phosphate-conjugated anti-digoxigenin Fab fragments and staining to detect enzyme activity. Left panels show results of hybridization with sense-control and right panels with Esk antisense probe.

Figure 9 is a photographic representation of Esk expression in sections of embryoid bodies and selected mouse tissues. Results of hybridizations with sense-control probe are shown in the left panels and with Esk antisense probe in the middle and right panels. (A) Embryoid bodies differentiated *in vitro*. (B) Adult thymus. (C) Adult renal cortex. (D) Day 18 embryo skin. Abbreviations: LP, low power; HP, high power.

Figure 10 is a photographic reprsentation showing binding of ligand to Esk chip in a biosense assay for binding to a potential Erk ligands. (L1 to L7) controls a FC, L3-FLAG, L7-FLAG and binding to HEK.

Single and three letter abbreviations for amino acid residues are used in the specification and are defined in Table 2.

TABLE 2

Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any residue	Xaa	X

EXAMPLE 1
ES CELL CULTURES

The murine 129/Sv-derived ES cell line, W9.5 was routinely passaged on underlays of irradiated embryonic fibroblasts in Dulbecco's modified Eagle medium supplemented with 1000 units/ml of LIF (AMRAD Operations Pty Ltd, Melbourne, Victoria, Australia), 10^{-4} M 2-mercaptoethanol and 15% v/v fetal calf serum. Cultures were incubated in a 10% v/v CO_2 atmosphere at 37°C. In preparation for the studies described below, ES cells were subcultured into delatinized flasks and four passages without a feeder layer were performed to deplete the embryonic fibroblasts. In some of these cultures, LIF was withdrawn 11 days prior to harvesting the cells, to allow differentiation into embryoid bodies (29). Control cultures of embryonic fibroblasts alone were also performed.

EXAMPLE 2
RT-PCR

Prior to RNA extraction, cultures of undifferentiated ES cells were disrupted with trypsin and washed in phosphate buffered saline. Cell pellets were resuspended in guanidine isothiocyanate denaturing buffer and total RNA was extracted using organic solvents (30). cDNA was then synthesized using 1 μg of total RNA, an aoligo(dT) primer and AMV reverse transcriptase (Promega). Four degenerate PCR primers were derived from three regions of sequence which are relatively conserved across the Eph subfamily (18)[Figure1}. Sense primers P1or P2 were used with antisense primer P4 to amplify the Es cell cDNA. Reactions were carried out in a 30μl volume containing 50 mM KC1, 10mM Tris.HC1 (pH 8.3), 1.25 mM $MgCl_2$, 0.2 mM each dNTP, 2.5 units of Taq polymerase (Perkin Elmer), 30 pmol of each promer and 3μl of the ES cell cDNA synthesis reaction. In another experiment, mRNA was directly extracted from ES cells with oligo9dT)-coated magnetic beads (Dynal; Oslo, Norway) and cDNA was synthesized from 1 μg of mRNA using an oligo(dT) primer and Superscript II reverse trasnscriptase (Life Technologies). Half of the reverse transcription reaction was amplified using primers P1 and P4, with other reaction

coditions identical to those described above. PCR products were electrophoretically separated, purified with the Geneclean II Kit (Bio 101 Inc) and then subjected to a second round of PCR. Products initially amplified using primers P1 and P4 were reamplified with the same primers, while those amplified with P2 and P4 were reasmplified with P2 and P3. All reactions were carried out in a PTC-100 Programmable Thermal Cotroller (MJ Research Inc) emplying programs specific to each primer combination, as follows: 1min at 95°C, 2 min at 70°C and 3 min at 72°C for 30 cycles (primers P1 and P4); 1 min at 95°C, 1 min at 51°C and 1 min at 72°C for 35 cycles (primers P2 and P4); 1 min at 95°C, 1 min at 41°C and 1 min at 72°C for 35 cycles (primers P2 and P3).

EXAMPLE 3

CLONING AND SEQUENCING

Reamplified, gel-purified PCR fragments were cloned into the *Sma*I site of pUC18 using the SureClone Ligation Kit (Pharmacia). A ^{32}P-labelled probe was synthesized from a cloned cDNA fragment of Esk. This was used to screen 8 x 10^5 clones from a mouse liver cDNA library constructed in λZAP. (Stratagene), under high stringency conditions. Secondary screening of selected primary positive plaques was used to identify clones with the largest inserts. Recombinant clones were sequences with the Taq DyeDeoxy terminartor Cycle Sequencing Kit (Appleid Biosystems), using a Perkin Elmer GeneAmp PCR System 2400 to perform the sequencing reactions and an Applied Biosystems 373 DNA Sequencer for their subsequent analysis. Sequences were compared against sequence databank entries with the FASTA sequence analysis program. A diagrammatic representation of Esk clones is shown in Figure 5.

EXAMPLE 4

NORTHERN BLOTS

Oligo(dT)-cellulose (Pharmacia) as used to isolate poly(A)$^+$ RNA from total derived from ES cells, embryoid bodies differentiated *in vitro* from ES cells. Embryonic fibroblasts, day-12 mouse embryos and adult mouse tissues. The poly(A)-selected samples (5μg of each) were electrophoresed through 1.2% w/v agarose gels containing 2.2 M formaldeyde and

transferred to nylon membrances (Zeta-Probe, Bio-Rad). The resulting Northern blots were probed with cDNA inserts from selected ES cell recombinants cloned by the methods described earlier. Sequence analysis (see below) had revealed that a partial cDNA of murine Eck (20) was present in the clone designated 35C4 and that Mek4 (7) cDNA was present in clone 35C11. Furthermore, an apparently novel sequence was ocntained in clone 35C15. Inserts were digested from these three clones with the restriction enzymes *Eco*RI and *Xba*I, and used as templates for synthesizing ^{32}P-lebelled probes witht he Prime-It II Random Primer Labelling Kit (Stratagene). A labelled propbe was also made from glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA. The Northern blots were intially hybridized to the 35C15 probe. Subsequently the membrane containg RNA from ES cells, embryoid lodies and embyronic fibroblasts was rrprobed with the Eck and Mek4 probes. Between each reprobing, hybridized probe was stripped from the membrane by pouring on boiling 0.1% w/v SDS and cooling to room temperature. Exposure to X-ray film overnight confirmed effective removal of the probe. Hybridisation to the GAPDH probe was performed last. In all cases, hybridisations took place in 50% v/v formamide at 42°C and washses were performed under stringent conditions, with the final wash at 65°C in 0.1 x SSC and 0.1% w/v SDS (SSC is 0.15 M NaCl and 0.015 m Na citrate, pH 7.6). Autoradiograps were exposed at -70°C.

EXAMPLE 5

SOUTHERN ANALYSIS

A ^{32}P-labelled Esk probe was synthesised from a PCR fragment amplified from Esk cDNA using degenerate kinase-domain primers described in Example 2. The fragment included sequence from bases 2098-2437 of Esk. Probe was hybridized to a geomic Southern blot made using standard techniques with DNA extractex from the murine embryonic stem cell line W9.5 and digested with the panel of enzymes showin in Figure 7. Hybridisation was performed in 50% formamide, 10x Denhardt's solutioN (35), 50mM Tris HCI (pH7.5), 1.0 < NaCl, 2.24 mM tetrasodium pyrophosphate, 1% w/v SDS, 10% w/v dextran sulfate and 0.1 mg/ml sheared heat-denatured herring sperm DNA. The final wash was in 0.1xSSC/0.1% w/v SDS at 65°C (SSC is 0.15 M sodium chloride, 0.015 M sodium ctrate,

pH 7.6). An autoradiograph was exposed at 70°C for eight days> The filter was subsequnetly stripped using 0.4 M sodium hydroxide and removal of the probe was confirmed by exposure to film overnight.

A ^{32}P-labelled Eph probe was synthesised from a cloned cDNA fragment isolated by RT-PCR from human bone marrow cDNA, using the same degenerate kinase-domain primers that generated the Esk probe. The cloned cDNA included sequence from bases 2212-2553 of human Eph. Probe was hybridized to the stripped Southern blot, with hybridization conditions and washes identifical to those used with the Esk probe. An autoradiograph was exposed at -70°C for seven days.

EXAMPLE 6

In vitro DIFFERENTIATION OF EMBRYOID BODIES

ES cells from the W9.5 line were induced to undergo differentiation by passaging them in bacterial-grade Petri dishes without an embryonic fibroblast feeder layer and in the absence of LIF. Embryoid bodies were harvested after 7-10 days of development (39).

EXAMPLE 7

PREPARATION OF WHOLE-MOUNTED AND SECTIONED TISSUE

CBA mouse embryos for whole-mount studies were collected 8-10 days following observation of the coital plug and a more accurate age was determined by reference to a standard atlas of mouse development (40). After opening the brain ventricles and heart chambers to prevent trapping of probe, the embryos were fixed in 4% v/v paraformaldehyde. Embryoid bodies for sections were set in 1% w.v agar, to which Bouin's fixative was added. Other tissues for sectioning, including day 18 CBA embryos and thymus, liver, kidney and adrenal gland from six-week-old BALB/c mice, were dissected free and fixed in 4% v/v paraformaldehyde. Sections were cut at a thickness of 5 μm after dehydration in ethanol and embedding in paraffin.

EXAMPLE 9

In situ HYBRIDIZATION PROCEDURE

The hybridization of digoxigenin-labelled riboprobes to whole-mounted specimens was performed using methods described in detail elsewhere (41), except that 2mM levamisole was included in the washes prior to colour development to inactivate endogenous alkaline phosphatases. Tissue sections were first soaked in histolene (Histo Labs) to remove paraffin and then rehydrated through decreasing concentrations of methanol in PBS. Refixation in 4% v/v paraformaldehyde and 0.2% v/v glutaraldehyde was then performed. Subsequent steps were similar to those of the whole-mount procedure.

EXAMPLE 10

Eph-SUBFAMILY MOLECULES EXPRESSED BY ES CELLS

Primers P2 and P4 were expected to amplify approximately 350 bp from the catalytic domain of Eph-subfamily molecules. In contrast, it was anticipated that primers P1 and P4 would amplify about 2.1 kb, including much of the extracellular and intracellular domains (Figure 1). RT-PCR was initially performed on total RNA derived from ES cells. Reactions using primers P2 and P4 amplified a band of the expected size, which reamplified with primers P2 and P3. However, no PCR product of the anticipated size was observed when primers P1 and P4 were used. Subsequently, when magnetically-separated poly(A)$^+$ RNA was substituted for total RNA, reactions with primers P1 and P4 successfully amplified a 2.1 kb product. Cloning of the 350 bp product resulted in four recombinants containing Eph-subfamily sequences (Table 3). These were highly homologous (>97%) to either Sek (9), Nuk (11) or Eck (20) - members of the Eph subfamily previously isolated from murine sources. When the 2.1 kb product was cloned, five recombinants containing sequences of this subfamily were identified. Four of these showed high levels of homology with either murine Eck or Mek4 (7), but one clone (35C15) contained 1604 bp of sequence which appeared novel after comparative databank analysis. The novel molecule was termed embryonic stem cell kinase or Esk.

A diagrammative representation of Esk clones is shown in Figure 5 and the sequence of the Esk cDNA is shown in Figure 2.

EXAMPLE 11
ISOLATION OF A FULL-LENGTH Esk CLONE

The Esk cDNA was used to screen a mouse liver cDNA library and identified twenty-four positive clones. One clone containing the largest insert size was analysed in detail and found to include the complete coding region of Esk (Figure 2). This clone contained a single open reading frame that encoded 977 amino acids, without an initiation codon occuring in a context consistent with the Kozac rules for translation start sites (42). Amino acids 1-26 conformed to the predicted sequence for a signal peptide (43). A sequence of predominantly hydrophobic residues at positions 549-569 were consistent with a transmembrane domain, potentially dividing the mature Esk protein into extracellular and intracellular portions. Within the putative extracellular domain were 20 highly-conserved cysteine residues (indicated by circles in Figure 2) and two fibronectin type III repeats which are hallmarks of the Eph subfamily (42). In the putative intracellular domain, motifs typical of a protein-tyrosine kinase were seen (31). This included the GXGXXG ATP-binding motif (residues 632-637) and the associated invariant lysine at position 657, along with motifs indicating substrate specificity for tyrosine (DLAARN, residues 750-755; and PIRWTAPE, residues 790-797). Finally, residues 60, 339, 415 and 479 are potential sites of N-liked glycosylation. Sequence database searching confirmed that the closest known Esk homologue was human Eph, with an overall amino acid sequence identity of 84.4%.

To confirm the localization of Esk, 135 grains over good-quality Chromosomes 6 were plotted onto the accurate idiogram of Evans (38). In this plot, the two tallest peaks of grains were again over bands 6B1 and 6B2 (Figure 6); together, these two peaks contained 56% of the total grains over Chromosome 6. The localization of the Esk gene to bands B1 and B2 of mouse Chromosome 6 was therefore confirmed by both sets of data.

EXAMPLE 12
ANALYSIS OF THE RELATIONSHIP BETWEEN Esk AND Eph

To determine whether Esk was the closest homologue of Eph in the mouse, a Southern blot of mouse genomic DNA digested with different restriction enzymes was sequentially hybridized to Esk and Eph probes. The probes were derived from exactly corresponding regions of their respective coding sequence. As shown in Figure 7, both probes recognized fragments of identical size with all restriction enzymes tested.

EXAMPLE 13
ESK EXPRESSION

Northern analysis of Esk revealed expression of a 4.2 kb transcript in day-12 mouse embryo and adult mouse thymus, liver, kidney, lung and placenta (Figure 3). Faint bands, approximately 6.0 kb in size, were also observed in the liver, kidney and lung samples. This may be due to alternatively spliced transcripts, although weak hybridization to related molecules cannot be excluded. No signal was detected from lymph node, spleen, heart, brain or skeletal muscle.

EXAMPLE 14

COMPARATIVE EXPRESSION OF Esk, Mek4 AND Eck IN THE ES CELL CULTURES

To determine whether expression of Eph-subfamily molecules occurred in ES cells at a significant level and to investigate potential changes in expression as ES cells were differentiated *in vitro*, a Northern blot containing ES cell and embryoid body RNA was sequentially hybridized to Esk, Mek4 and Eck probes. Embryonic fibroblast RNA was included as a control on this blot, because some fibroblast contamination of the ES cell and embryoid body samples could not be excluded. As shown in Figure 4, the Esk probe hybridized to all three samples, approximately in proportion to the amount of RNA present. This result indicates significant levels of Esk expression in ES cells and embryoid bodies, and could not be accounted for by fibroblast contamination alone. In contrast, expression of Mek4 was barely detectable in the ES cell cultures. A 9.5 kb Mek4 transcript was expressed by the embryonic fibroblasts, however, suggesting that the faint bands seen in the ES cell lane may be due to contaminating fibroblast RNA. Finally, the Eck probe hybridized to all three samples, but expression was relatively greater in the undifferentiated ES cells. Transcripts were of slightly different sizes in the different lanes.

EXAMPLE 15

EXPRESSION

In situ studies with digoxigenin-labelled ESK riboprobes were used to analyse expression using manufactuers protocols (Boehringer-Mannheim *In situ* hybridisation manual). Whole mount preparations of early (D8-12) mouse embryos show that ESK is expressed in the developing fore-gut and branchial arches. Later embryos were sectioned and analysed by *in situ* hybridisation. In these stages expression was noted in the thymus, basal layer of the skin, lung and in the developing eye. Similar studies were performed in tissue sections of adult tissues. In the adult animal expression was also seen in the thymus, as expected from Northern blot analysis, but was shown to be localised in the thymic epithelial cells. There was also detectable expression in the proximal and distal tubules of the kidney, the adrenal cortex and in testis. While expression was expected in other sites from the Northern analysis results, no

signal greater than background was evident, perhaps suggesting low level expression of RNA degradation during processing.

Expression in epithelial but not in neural tissues confirms the impression that ESK is primarily involved in epithelial development. The expression in thymic epithelial cells is of particular interest as thee cells have been shown to be involved in thymocyte selection and hence in development of T cell immune competence.

EXAMPLE 16
ANALYSIS OF Esk EXPRESSION BY *In situ* HYBRIDIZATION

The expression of Esk was investigated with *in situ* hybridization in embryoid bodies differentiated *in vitro*, mouse embryos and selected adult mouse tissues. These experiments were performed using two pairs of antisense and sense-control riboprobes derived from non-overlapping regions of Esk sequence. Both antisense probes gave similar results. No specific staining was detected in any of the tissues hybridized to the sense-control probes with the exception of small intestine, where high levels of endogenous alkaline phosphatase activity resulted in false-positive staining of the epithelium. This occured despite the inclusion of levamisole in the post-hybridization washes.

EXAMPLE 17
EMBRYOID BODIES

The strong expression of Esk in ES cells and embryoid bodies observed with Northern analysis (Example 13) prompted investigation into whether expression was differentially regulated even at this early stage. In the current study *in situ* hybridization experiments were performed on embryoid bodies resulting from the differentiation of ES cells for 7-10 days *in vitro*. Differentiation was induced by growing the cells without a fibroblast feeder layer and in the absence of exogenous leukemia inhibitory factor (LIF), as previously described (39). Specific binding of antisense probe, indicating Esk mRNA expression, was

observed in both whole-mounted and sectioned embryoid bodies. In the whole-mounts, staining appeared strongest in the central region of embryoid bodies (Figure 8A). This distribution was confirmed by the sections. As shown in Figure 9A, although Esk was expressed by most embryoid-body cells, an amorphous layer visible near the surface at high magnification delineated an outer rind of cells that did not stain. The latter cells have been shown by others to correspond to primitive endoderm, whereas the cells within the interior are primitive ectoderm in type (45; 46). These populations of cells are separated by a layer of basement membrane-like material secreted by the endodermal cells.

EXAMPLE 18
EMBRYOS

To investigate Esk expression during later development, whole-mount preparations of embryos at days 8, 8.5, 9 and 9.5 of gestation were examined. The pattern of expression was similar in all embryos analysed and a representative specimen at day 9.5 of development is shown in Figure 8B. This demonstrates that Esk was predominantly expressed in ventral structures, including the branchial arches and the region containing the developing gut and its associated epithelial outgrowths. Expression was also observed in the region of the optic vesicle and there was lighter staining of the somites and other structures. No significant staining of the central nervous system was seen, which contrasts with the predominantly neural expression of many other Eph-subfamily receptors. Sections of day 18 embryos were also studied by *in situ* hybridization. These showed no specific staining for Esk mRNA in the majority of tissues. However, day 18 embryonic skin demonstrated positive staining in the deepest layers of the epidermis and in the developing hair follicles (Figure 9D). It is noteworthy that these are the regions of skin associated with active cellular proliferation and migration of cells into other layers.

EXAMPLE 19
ADULT TISSUES

Northern analysis (Example 13) indicated that Esk is expressed in a variety of non-neural adult tissues. To define the specific cells containing Esk mRNA, *in situ* hybridisation experiments were performed on sections of some of these tissues. In thymus, Esk expression was observed in aggregates of large cells with abundant cytoplasm, located predominantly in the medulla (Figure 9B). Morphologically, these cells were consistent with thymic epithelia. No staining of thymocytes was observed. In kidney, Esk expression was localized to the epithelium of proximal and distal convoluted tubules (Figure 9C). Glomeruli and the interstium did not stain. Esk was also expressed in the epithelial cells of the adrenal cortex. In sections of liver, small foci of hepatocytes stained positively, but the majority of the tissue was negative. As discussed earlier, no assessment of Esk expression in small intestine was possible, because of endogenous alkaline phosphatase activity.

EXAMPLE 20
CHROMOSOMAL LOCALIZATION

To determine the chromosomal localization of the Esk gene, a tritiated probe was made from the 1.6 kb cloned PCR fragment and hybridized *in situ* to preparations of mouse metaphase chromosomes. This resulted in a significant accumulation of silver grains over bands B1 and B2 of mouse Chromosome 6. Out of an initial score of 345 grains for all chromosomes, 36.8% of grains were over the proximal half of Chromosome 6. Background grains were distributed over the other chromosomes with 5 grains over distal Chromosome 15 to tallest secondary peak observed. This compared to 59 grains over band 6B2 and 28 over 6B1.

EXAMPLE 21
PREPARATION OF SECRETED RECOMBINANT Esk PROTEIN

1. MATERIALS AND METHODS

Cell lines

COS cells and CHO cells were propagated in RPMI containing 10% v/v heat-inactivated FCS in a humidified 10% v/v CO_2 atmosphere at 37°C. The COS cells were stably transfected with the polyoma large T antigen, permitting replication of vectors containing an SV40 origin of replication. For experiments involving selection of CHO cells cotransfected with the pSV2neo vector, a concentration of 600 μg/ml of G418 (Gibco BRL) in RPMI/1-% v/v FCS was used.

Assembly of an Esk-IgG Fc Construct

cDNA sequence was amplified from Esk using a 5′ primer derived from sequence beginning five bases upstream of the translation start site and a 3′ primer from sequence immediately upstream of the transmembrane domain. Custom *BglII* sites were included in both primers and a splice donor sequence was added to the 3′ primer. To minimize the possibility of PCR-induced errors, amplifications were performed using recombinant (Stratagene). The reaction was carried out in a 50 μl volume containing 1x cloned *Pfu* buffer, 0.2 mM each dNTP, 2.5 units cloned *Pfu* polymerase, 0.2 μM each primer and approximately 250 ng of pBluescript plasmid containing the full-length Esk clone, and was performed in a Perkin Elmer GeneAmp 2400 PCR machine. PCR amplification conditions were: 30 seconds at 95°C, 30 seconds at 55°C and 2 minutes and 30 seconds at 72°C for 5 cycles; 30 seconds at 95°C and 3 minutes at 72°C for 15 cycles. Amplification products were digested with *BglII* and ligated into the unique *Bam*HI cloning site of the plg-BOS expression vector. This plasmid is a pEF-BOS derivative which includes a genomic fragment of human IgG1 (containing exons encoding the hinge, CH2 and CH3 domains) downstream of the cloning site. Expression of this construct was expected to produce a polypeptide of approximately 90 kD in mass, which should become a divalent molecule of approximately 180 kD after the formation of cystine bonds between the hinge regions. The constructs were cloned in DH10 B *E.coli* cells and clones containing correctly oriented inserts were identified and DNA prepared and checked by DNA sequencing.

COS Cell Transfections

Five micrograms of expression plasmid prepared using a QIAGEN maxiprep kit was transiently transfected into COS cells using a standard DEAE-dextran method (35). A control was also performed using identical conditions except no DNA was added. After three days of incubation, the culture supernatant was removed for analysis. Cells lysates were also obtained using 1% v/v Triton X-100 in 10mM Tris HCI (pH 7.4) and 150 mM NaCI.

CHO Cell Transfection and Subcloning

Ten micrograms of the Esk-IgG Fc expression construct and 1 μg of pSV2neo G418-resistance plasmid were cotransfected into 2x10^7 CHO cells by electroporation. This was performed in 0.5 ml of PBS in a 4 mm sterile cuvette using 270 V and 960 μFD on a BioRad Gene Pulser. Cells were incubated in 15 cm tissue culture dishes for 24 hours before selection with G418 was commenced. After the development of visible clones, these were subcloned into 24-well plates and the supernatant harvested for analysis when the cells were confluent.

Western Blots

Five microlitres of supernatant or cell lysate from the transfections described above was resolved by SDS polyacrylamid gel electrophoresis and transferred to a nitrocellulose membrane using established methods (35). Samples from the transfections were electrophoresed under both reducing and non-reducing conditions. Western blots were subsequently blocked in 5% w/v skimmed milk powder and 0.5% v/v Tween-20. After blocking as both a rabbit anti-human Ig antibody (HRP-conjugated) and a rabbit anti-Esk peptide polyclonal serum (see below) the antibodies were used to probe the blots with a secondary layer consisting of an HRP-conjugated donkey anti-rabbit Ig antibody (Amersham) applied in the latter case. Detection was with the ECL kit (Amersham).

Production of An Anti-Esk peptide Polyclonal Serum

A peptide was synthesized from the first 31 amino acids of the predicted mature protein encoded in the Esk sequence. This was coupled to keyhole limpet hemocyanin (KLH) and 200 μg of the conjugate was emulsified in complete Freund's adjuvant and injected into a New Zealand white rabbit *via* multiple subcutaneous sites. A second dose of antigen was administered in incomplete Freund's adjuvant after four weeks and serum was collected two weeks later. The serum was used at a dilution of 1:100 to probe Western blots.

Immunohistochemistry

CHO cells permanently transfected with an Esk and control CHO cells were grown in chamber slides, fixed in PBS containing 1%v/v paraformaldehyde for 30 minutes and permeabilized with 100% v/v methanol. After blocking in PBS containing 2% v/v FCS and 2% v/v goat serum, the cells were incubated with either a 1:100 dilution of the rabbit anti-Esk antiserum described in the previous section or with preimmune serum as a control. In a second set of controls, 40 μg of Esk peptide coupled to KLH was added with the antiserum. A donkey anti-rabbit Ig antibody conjugated to HRP was used as the secondary antibody in all cases and bound antibody was detected with diaminobenzidine (Dako).

Protein A affinity purification

The following method was used for protein A-Sepharose affinity chromatography. The column was pre-eluted with pH 3 0.1 M acetate buffer and re-equilibrated with PBS. The sample was applied and then the column washed to baseline with PBS. The sample was eluted with acetate buffer and peak fractions collected in tubes containing 1/10 vol of 1M tris HCI pH8. The column was re-equilibrated with PBS/azide for storage.

Biosensor studies

The studies were carried on a BIAcore 2000 (Pharmacia) using the manufacturers protocols. Individual channels of a biosensor CM chip were derivatised with ESK-Fc or with soluble HEK (47). Samples of LERK-2, -2, 03, -4, -5, -7 as Fc fusion proteins were analysed at 10μg/ml. Single chain forms of LERK-3 and -7 expressed as FLAG™ (Kodak) constructs were produced by the inventors and were analysed at 2μg/ml. In each case a parallel sample was run in the presence of excess ESK-Fc. The results are presented at the difference between these responses (ie. the ESK-specific component) for each test sample and are shown in Figure 10.

Polyclonal anti-Esk-Fc serum

The purified ESK Fc protein (100 μg) was homogenised in complete Freund's adjuvant and administered subcutaneously at multiple sites. Animals were immunised every 14 days and bled 10 days after each injection. To purify the specific component the antiserum was exhaustively absorbed on a human immunoglobulin affinity column and then applied to an ESK Fc affinity column. the specific antibody was eluted with 0.2 M glycine HCI pH 2.3, quickly neutralised and concentrated to 0.5-1.0 mg/ml.

2. RESULTS

Analysis of the Rabbit Anti-Esk Polclonal Serum

A polyclonal serum raised against an Esk peptide was initially tested by probing a Western blot containing recombinant Esk and Hek. The antibody recognized Esk specifically, with bands of the expected size appearing in both the full-length and truncated Esk samples and no specific bands in the Hek samples. When the anti-Esk serum was used for immunochemistry, the cytoplasm of CHO cells transfected with the Esk construct stained strongly. The specificity of this staining for Esk was confirmed by several controls. These included incubations of Esk-transfected cells with preimmune serum and of control CHO cells with the antiserum. Only background staining was observed in either case. In addition, Esk peptide as observed to inhibit staining of the transfectants by the antiserum.

Transfection of the Esk-IgG Fc and CH48-IgG Fc Constructs

Western blots of the supernatants of COS cells transfected with the Esk-IgG construct. Specific bands of the expected size were detected in the transfected samples. Both the anti-Ig antibody and the anti-Esk antiserum gave positive signals. the Esk-Fc construct and a control construct being a fusion between CD48 and IgG Fc were used to generate permanently-transfected CHO cells lines. Western blots of conditioned medium from the ESK-Fc clones revealed a similar pattern to that of the COS cell transfections. The CH48 Fc band was somewhat smaller. Lines which secreted very high levels of the fusion protein were selected.

Purification of ESK & CD48 Fc proteins

High level expressing clones were expanded to 1 litre cultures in RPMI 1640 supplemented with 2.5% v/v FCS which had been absorbed on protein A-Sepharose beads to remove all protein A-binding bovine immunoglobulin. The cultures were allowed to grow to confluence and the conditioned medium removed, centrifuged to remove cellular material and

concentrated 10-fold. The medium was applied to a protein A Sepharose column as above. Purified protein was analysed by SDS-PAGE to confirm purity. In each case a single band was obtained on SDS-PAGE under reducing conditions. N terminal sequencing of the ESK-Fc protein band gave a single un-equivocal sequence:

GluGluValThrLeuMetAspThr [SEQ ID NO:9]

which matches residues 27-34 of the amino acid sequence of ESK derived from the nucleotide sequence.

Binding of LERK proteins to ESK Fc

The samples show the expected pattern of binding to soluble HEK. There is also clear evidence of binding of divalent LERK-1, -3 and -4 Fc samples to the Esk channel. However, the predicted maximal binding would have given much larger responses (as seen on the HEK chip) suggesting that the binding is relatively weak. This is highlighted in the case of LERK-3 by the failure to defect any binding of monomeric ligand to Esk.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

TABLE 3

Eph-subfamily cDNA clones isolated from ES cells by RT-PCR

Clone designation	Closest homologue	Homology (%)
	Primers P2 and P4*	
25C5	Sek	97.8
33C1.1	Nuk	97.2
33C1.2	Eck	99.1
33C1.5	Eck	100
	Primers P1 and P4*	
35C4	Eck	97.2
35C6	Mek4	97.3
35C10	Mek4	99.7
35C11	Mek4	99.7
35C15	Eph	83

Homologues were identified by screening nucleic acid databanks with the FASTA program. Sek (11), Nuk (15), Eck (21) and Mek (8) are murine Eph-subfamily molecules; Eph (3) is a human molecule.

* Refers to the primers used in the initial amplification during RT-PCR.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: (OTHER THAN US) AMRAD OPERATIONS PTY LTD
(US ONLY):- Andrew Wallace BOYD and Jason Lickliter

(ii) TITLE OF INVENTION: A NOVEL RECEPTOR-TYPE TYROSINE KINASE AND USE THEREOF

(iii) NUMBER OF SEQUENCES: 9

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 20-DEC-1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: AU PN7277/95
(B) FILING DATE: 22-DEC-1995

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3309 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2931

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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AACATATGTC CCCAAAGAGT TTAAAGCTGA GACCTTCACC TTCCACTCTG ATATCTGCAC      -175

ACTTCCAGAG AAGGAGAAGC AGATTAAGAA ACAAACGGCT CTTGCTGAGC TGGTGAAGCA      -115

CAAGCCCAAG GCTACAGCGG AGCAACTGAA GACTGTCATG GATGACTTTG CACAGTTCCT      -55

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GAC ACA AGC ACA GCA CAA GGA GAG CTG GGC TGG CTT CTG GAT CCC CCA       144
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Tyr Met Tyr Gln Asp Cys Pro Ile Gln Glu Gly Gly Asp Thr Asp His
 65                  70                  75                  80

TGG CTT CGC TCC AAT TGG ATC TAC CGC GGA GAG GAA GCT TCA CGC ATC    288
Trp Leu Arg Ser Asn Trp Ile Tyr Arg Gly Glu Glu Ala Ser Arg Ile
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Tyr Val Glu Leu Gln Phe Thr Val Arg Asp Cys Lys Ser Phe Pro Gly
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    130                 135                 140

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    210                 215                 220

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Leu Val Pro Val Gly Gln Cys Gln Cys Glu Pro Gly Tyr Glu Glu Ser
260 265 270

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Ser Gly Asn Val Gly Cys Thr Ala Cys Pro Thr Gly Phe Tyr Arg Val
275 280 285

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290 295 300

GAG TCT GAG GGG TCT ACC ATC TGT ACC TGT GAG AAT GGA CAT TAT CGA 960
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305 310 315 320

GCC CCT GGG GAG GGT CCC CAG GTA GCA TGC ACA CGT CCC CCA TCG GCT 1008
Ala Pro Gly Glu Gly Pro Gln Val Ala Cys Thr Arg Pro Pro Ser Ala
325 330 335

CCC CAA AAT CTG AGC TTC TCC ACA TCA GGG ACT CAA CTC TCC CTG CGC 1056
Pro Gln Asn Leu Ser Phe Ser Thr Ser Gly Thr Gln Leu Ser Leu Arg
340 345 350

TGG GAG CCC CCC AGA GAT ACA GGG GGA CGC CAT GAT ATC AGA TAC AGC 1104
Trp Glu Pro Pro Arg Asp Thr Gly Gly Arg His Asp Ile Arg Tyr Ser
355 360 365

GTG GAG TGC TTG CAG TGT CGG GGC ATT GCA CAG GAT GGG GGT CCC TGC 1152
Val Glu Cys Leu Gln Cys Arg Gly Ile Ala Gln Asp Gly Gly Pro Cys
370 375 380

CAA CCC TGT GGA AAA GGT GTG CAC TTT TCC CCG GCT GCT TCC GGG CTC 1200
Gln Pro Cys Gly Lys Gly Val His Phe Ser Pro Ala Ala Ser Gly Leu
385 390 395 400

ACC ACA TCT ACC GTG CAA GTG CAA GGC CTC GAG CCT TAC GCC AAC TAC 1248
Thr Thr Ser Thr Val Gln Val Gln Gly Leu Glu Pro Tyr Ala Asn Tyr
405 410 415

ACA TTT ACC GTC AAA TCC CAA AAC AGA GTG TCA GGA CTG GAC AGT TCC 1296
Thr Phe Thr Val Lys Ser Gln Asn Arg Val Ser Gly Leu Asp Ser Ser
420 425 430

AGC CCT AGC AGC GCC TCC CTC AGT ATC AAC ATG GGG CAC GCA GAG TCA 1344
Ser Pro Ser Ser Ala Ser Leu Ser Ile Asn Met Gly His Ala Glu Ser
435 440 445

CTC TCT GGC CTG TCA CTG AAG CTG GTG AAG AAA GAA CCG AGG CAG CTG 1392
Leu Ser Gly Leu Ser Leu Lys Leu Val Lys Lys Glu Pro Arg Gln Leu
450 455 460

GAG CTG ACT TGG GCA GGG TCC CGA CCC CGA AAT CCT GGA GGG AAT CTG 1440
Glu Leu Thr Trp Ala Gly Ser Arg Pro Arg Asn Pro Gly Gly Asn Leu
465 470 475 480

AGC TAT GAG CTG CAC GTG CTG AAT CAG GAC GAA GAA TGG CAC CAG ATG 1488
Ser Tyr Glu Leu His Val Leu Asn Gln Asp Glu Glu Trp His Gln Met
485 490 495

GTG TTG GAA CCC AGG GTC TTG CTG ACA AAA CTT CAG CCA GAT ACC ACA 1536
Val Leu Glu Pro Arg Val Leu Leu Thr Lys Leu Gln Pro Asp Thr Thr
500 505 510

TAC ATT GTC AGA GTG CGA ACA CTG ACC CCA CTG GGG CCT GGC CCT TTC 1584
Tyr Ile Val Arg Val Arg Thr Leu Thr Pro Leu Gly Pro Gly Pro Phe
515 520 525

TCC CCT GAC CAT GAG TTT CGG ACA AGC CCA CCA GTT TCC AGA AGC CTG 1632
Ser Pro Asp His Glu Phe Arg Thr Ser Pro Pro Val Ser Arg Ser Leu
530 535 540

ACC GGA GGA GAG ATT GTG GCC GTC ATC TTT GGA TTG CTG CTT GGA ATA 1680
Thr Gly Gly Glu Ile Val Ala Val Ile Phe Gly Leu Leu Leu Gly Ile
545 550 555 560

GCT CTG CTG ATC GGG ATT TAT GTC TTC CGT TCA AGG AGA GGC CAG AGA 1728
Ala Leu Leu Ile Gly Ile Tyr Val Phe Arg Ser Arg Arg Gly Gln Arg
565 570 575

CAG AGA CAG CAG AGG CAG CGT GAA CGC ACC ACC AAT GTC GAT CGA GAG 1776
Gln Arg Gln Gln Arg Gln Arg Glu Arg Thr Thr Asn Val Asp Arg Glu
580 585 590

GAC AAG CTG TGG CTA AAA CCC TAT GTG GAC CTC CAG GCC TAT GAG GAC 1824
Asp Lys Leu Trp Leu Lys Pro Tyr Val Asp Leu Gln Ala Tyr Glu Asp

595 600 605

CCT GCA CAG GGA GCC TTA GAC TTT GCC CAG GAA CTG GAC CCA GCC TGG 1872
Pro Ala Gln Gly Ala Leu Asp Phe Ala Gln Glu Leu Asp Pro Ala Trp
610 615 620

CTG ATT GTG GAC ACT GTC ATA GGA GAA GGG GAG TTT GGT GAA GTG TAT 1920
Leu Ile Val Asp Thr Val Ile Gly Glu Gly Glu Phe Gly Glu Val Tyr
625 630 635 640

CGG GGA GCC CTG AGA CTC CCC AGC CAA GAT TGC AAG ACT GTG GCC ATT 1968
Arg Gly Ala Leu Arg Leu Pro Ser Gln Asp Cys Lys Thr Val Ala Ile
645 650 655

AAG ACC TTG AAA GAT ACA TCC CCA GAT GGC TAC TGG TGG AAT TTC CTT 2016
Lys Thr Leu Lys Asp Thr Ser Pro Asp Gly Tyr Trp Trp Asn Phe Leu
660 665 670

CGA GAG GCA ACT ATC ATG GGC CAG TTC AAC CAC CCA CAC ATT CTA CGC 2064
Arg Glu Ala Thr Ile Met Gly Gln Phe Asn His Pro His Ile Leu Arg
675 680 685

CTA GAA GGT GTC ATC ACA AAA AGA AAG CCC ATC ATG ATC ATC ACA GAA 2112
Leu Glu Gly Val Ile Thr Lys Arg Lys Pro Ile Met Ile Ile Thr Glu
690 695 700

TTT ATG GAA AAT GGA GCC CTG GAT GCC TTT CTG AAG GAA CGG GAG GAC 2160
Phe Met Glu Asn Gly Ala Leu Asp Ala Phe Leu Lys Glu Arg Glu Asp
705 710 715 720

CAA CTA GCT CCT GGT CAG CTA GTG GCT ATG CTA CTG GGC ATA GCA TCA 2208
Gln Leu Ala Pro Gly Gln Leu Val Ala Met Leu Leu Gly Ile Ala Ser
725 730 735

GGC ATG AAC TGC CTC AGT GGC CAC AAT TAT GTC CAT AGA GAC CTG GCT 2256
Gly Met Asn Cys Leu Ser Gly His Asn Tyr Val His Arg Asp Leu Ala
740 745 750

GCC AGG AAC ATC TTG GTG AAT CAG AAC CTG TGC TGC AAG GTG TCT GAC 2304
Ala Arg Asn Ile Leu Val Asn Gln Asn Leu Cys Cys Lys Val Ser Asp
755 760 765

TTT GGC TTG ACC CGC CTC CTG GAT GAC TTT GAC GGC ACC TAT GAA ACC 2352
Phe Gly Leu Thr Arg Leu Leu Asp Asp Phe Asp Gly Thr Tyr Glu Thr
770 775 780

CAG GGA GGA AAG ATC CCC ATC CGA TGG ACA GCC CCA GAA GCT ATT GCC 2400
Gln Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ala
785 790 795 800

CAT CGG ATC TTC ACC ACA GCC AGT GAT GTG TGG AGC TTT GGG ATT GTA 2448
His Arg Ile Phe Thr Thr Ala Ser Asp Val Trp Ser Phe Gly Ile Val
805 810 815

ACG TGG GAG GTG TTG AGT TTT GGC GAC AAA CCC TAT GGG GAG ATG AGC 2496
Thr Trp Glu Val Leu Ser Phe Gly Asp Lys Pro Tyr Gly Glu Met Ser
820 825 830

AAC CAA GAG GTA ATG AAA AGC ATT GAA GAT GGG TAC CGG TTG CCC CCT 2544
Asn Gln Glu Val Met Lys Ser Ile Glu Asp Gly Tyr Arg Leu Pro Pro
835 840 845

CCT GTG GAC TGT CCT GCC CCT CTC TAT GAA CTC ATG AAG AAC TGC TGG 2592
Pro Val Asp Cys Pro Ala Pro Leu Tyr Glu Leu Met Lys Asn Cys Trp
850 855 860

GCT TAC GAT CGT GCC CGT CGA CCC CAC TTC CTC CAG CTG CAG GCA CAT 2640
Ala Tyr Asp Arg Ala Arg Arg Pro His Phe Leu Gln Leu Gln Ala His
865 870 875 880

CTG GAA CAG TTG CTT ACT GAC CCC CAT TCC CTA AGG ACA ATT GCC AAC 2688
Leu Glu Gln Leu Leu Thr Asp Pro His Ser Leu Arg Thr Ile Ala Asn
885 890 895

TTT GAC CCT AGG GTG ACC TTA CGC CTG CCC AGC CTG AGT GGC TCT GAT 2736
Phe Asp Pro Arg Val Thr Leu Arg Leu Pro Ser Leu Ser Gly Ser Asp
900 905 910

GGG ATC CCT TAT CGA AGT GTC TCT GAG TGG CTT GAA TCC ATA CGC ATG 2784
Gly Ile Pro Tyr Arg Ser Val Ser Glu Trp Leu Glu Ser Ile Arg Met
915 920 925

AAG CGC TAC ATC CTG CAC TTC CGT TCG GCT GGG CTG GAC ACC ATG GAG 2832
Lys Arg Tyr Ile Leu His Phe Arg Ser Ala Gly Leu Asp Thr Met Glu
930 935 940

TGT GTG CTG GAG CTG ACG GCT GAG GAC CTG ACG CAG ATG GGA ATA ACG 2880
Cys Val Leu Glu Leu Thr Ala Glu Asp Leu Thr Gln Met Gly Ile Thr
945 950 955 960

TTG CCA GGG CAC CAG AAA CGA ATT CTC TGC AGT ATT CAA GGA TTT AAG 2928
Leu Pro Gly His Gln Lys Arg Ile Leu Cys Ser Ile Gln Gly Phe Lys

965 970 975

GAC TGAGCATCCA CTGAAAAGAT GCTCCAGCCC TCTGCCTGCC TCCATTAGCA 2981
Asp

AGGACGGGGT ACAGTCAACT CCCTGGGCCT TTCCTCAGCC TACGAAATGT AGGCTATTGG 3041

TGCTGCTCCT GCCCAGTCAA TCAGAACTCT GCCTTTGAAC CAAGGAGCCT TTGTTTATAA 3101

AGGGGGTGGA TGGGTACAAG TGAAGGGGnC TGTGGGTGGG TTCTGGGGGA GGGTTTAATA 3161

nATACACTTA CATATGCATT ATCTATTTTT GTAAATAAAC AAGAGTTGAG TTTTAAAAAA 3221

AAAAAAAAAG GAATTCCTGC AGCCCGGGGG ATCCACTAGT TCTAGAGCGG CCGCCACCGC 3281

GGTGGAGCTC CAGCTTTTGT TCCCTTTA 3309

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 977 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Glu Arg Arg Trp Pro Leu Gly Leu Ala Leu Leu Leu Leu Leu Leu
1 5 10 15

Cys Ala Pro Leu Pro Pro Gly Ala Arg Ala Glu Glu Val Thr Leu Met
20 25 30

Asp Thr Ser Thr Ala Gln Gly Glu Leu Gly Trp Leu Leu Asp Pro Pro
35 40 45

Glu Thr Gly Trp Ser Glu Val Gln Gln Met Leu Asn Gly Thr Pro Leu
50 55 60

Tyr Met Tyr Gln Asp Cys Pro Ile Gln Glu Gly Gly Asp Thr Asp His
65 70 75 80

Trp Leu Arg Ser Asn Trp Ile Tyr Arg Gly Glu Glu Ala Ser Arg Ile

85 90 95

Tyr Val Glu Leu Gln Phe Thr Val Arg Asp Cys Lys Ser Phe Pro Gly
100 105 110

Gly Ala Gly Pro Leu Gly Cys Lys Glu Thr Phe Asn Leu Phe Tyr Met
115 120 125

Glu Ser Asp Gln Asp Val Gly Ile Gln Leu Arg Arg Pro Leu Phe Gln
130 135 140

Lys Val Thr Thr Val Ala Ala Asp Gln Ser Phe Thr Ile Arg Asp Leu
145 150 155 160

Ala Ser Asp Ser Val Lys Leu Asn Val Glu Arg Cys Ser Leu Gly His
165 170 175

Leu Thr Arg Arg Gly Leu Tyr Leu Ala Phe His Asn Pro Gly Ser Cys
180 185 190

Val Ala Leu Val Ser Val Arg Val Phe Tyr Gln Arg Cys Ala Glu Thr
195 200 205

Val His Gly Leu Ala His Phe Pro Asp Thr Leu Pro Gly Pro Gly Gly
210 215 220

Leu Val Glu Val Ala Gly Thr Cys Leu Ser His Ala Gln Ile Ser Leu
225 230 235 240

Gly Ser Ser Gly Thr Pro Arg Met His Cys Ser Pro Asp Gly Glu Trp
245 250 255

Leu Val Pro Val Gly Gln Cys Gln Cys Glu Pro Gly Tyr Glu Glu Ser
260 265 270

Ser Gly Asn Val Gly Cys Thr Ala Cys Pro Thr Gly Phe Tyr Arg Val
275 280 285

Asp Met Asn Thr Leu Arg Cys Leu Lys Cys Pro Gln His Ser Ile Ala
290 295 300

Glu Ser Glu Gly Ser Thr Ile Cys Thr Cys Glu Asn Gly His Tyr Arg
305 310 315 320

Ala Pro Gly Glu Gly Pro Gln Val Ala Cys Thr Arg Pro Pro Ser Ala
325 330 335

Pro Gln Asn Leu Ser Phe Ser Thr Ser Gly Thr Gln Leu Ser Leu Arg
340 345 350

Trp Glu Pro Pro Arg Asp Thr Gly Gly Arg His Asp Ile Arg Tyr Ser
355 360 365

Val Glu Cys Leu Gln Cys Arg Gly Ile Ala Gln Asp Gly Gly Pro Cys
370 375 380

Gln Pro Cys Gly Lys Gly Val His Phe Ser Pro Ala Ala Ser Gly Leu
385 390 395 400

Thr Thr Ser Thr Val Gln Val Gln Gly Leu Glu Pro Tyr Ala Asn Tyr
405 410 415

Thr Phe Thr Val Lys Ser Gln Asn Arg Val Ser Gly Leu Asp Ser Ser
420 425 430

Ser Pro Ser Ser Ala Ser Leu Ser Ile Asn Met Gly His Ala Glu Ser
435 440 445

Leu Ser Gly Leu Ser Leu Lys Leu Val Lys Lys Glu Pro Arg Gln Leu
450 455 460

Glu Leu Thr Trp Ala Gly Ser Arg Pro Arg Asn Pro Gly Gly Asn Leu
465 470 475 480

Ser Tyr Glu Leu His Val Leu Asn Gln Asp Glu Glu Trp His Gln Met
485 490 495

Val Leu Glu Pro Arg Val Leu Leu Thr Lys Leu Gln Pro Asp Thr Thr
500 505 510

Tyr Ile Val Arg Val Arg Thr Leu Thr Pro Leu Gly Pro Gly Pro Phe
515 520 525

Ser Pro Asp His Glu Phe Arg Thr Ser Pro Pro Val Ser Arg Ser Leu
530 535 540

Thr Gly Gly Glu Ile Val Ala Val Ile Phe Gly Leu Leu Leu Gly Ile
545 550 555 560

Ala Leu Leu Ile Gly Ile Tyr Val Phe Arg Ser Arg Arg Gly Gln Arg
565 570 575

Gln Arg Gln Gln Arg Gln Arg Glu Arg Thr Thr Asn Val Asp Arg Glu

580 585 590

Asp Lys Leu Trp Leu Lys Pro Tyr Val Asp Leu Gln Ala Tyr Glu Asp
595 600 605

Pro Ala Gln Gly Ala Leu Asp Phe Ala Gln Glu Leu Asp Pro Ala Trp
610 615 620

Leu Ile Val Asp Thr Val Ile Gly Glu Gly Glu Phe Gly Glu Val Tyr
625 630 635 640

Arg Gly Ala Leu Arg Leu Pro Ser Gln Asp Cys Lys Thr Val Ala Ile
645 650 655

Lys Thr Leu Lys Asp Thr Ser Pro Asp Gly Tyr Trp Trp Asn Phe Leu
660 665 670

Arg Glu Ala Thr Ile Met Gly Gln Phe Asn His Pro His Ile Leu Arg
675 680 685

Leu Glu Gly Val Ile Thr Lys Arg Lys Pro Ile Met Ile Ile Thr Glu
690 695 700

Phe Met Glu Asn Gly Ala Leu Asp Ala Phe Leu Lys Glu Arg Glu Asp
705 710 715 720

Gln Leu Ala Pro Gly Gln Leu Val Ala Met Leu Leu Gly Ile Ala Ser
725 730 735

Gly Met Asn Cys Leu Ser Gly His Asn Tyr Val His Arg Asp Leu Ala
740 745 750

Ala Arg Asn Ile Leu Val Asn Gln Asn Leu Cys Cys Lys Val Ser Asp
755 760 765

Phe Gly Leu Thr Arg Leu Leu Asp Asp Phe Asp Gly Thr Tyr Glu Thr
770 775 780

Gln Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Ala
785 790 795 800

His Arg Ile Phe Thr Thr Ala Ser Asp Val Trp Ser Phe Gly Ile Val
805 810 815

Thr Trp Glu Val Leu Ser Phe Gly Asp Lys Pro Tyr Gly Glu Met Ser
820 825 830

Asn Gln Glu Val Met Lys Ser Ile Glu Asp Gly Tyr Arg Leu Pro Pro
835 840 845

Pro Val Asp Cys Pro Ala Pro Leu Tyr Glu Leu Met Lys Asn Cys Trp
850 855 860

Ala Tyr Asp Arg Ala Arg Arg Pro His Phe Leu Gln Leu Gln Ala His
865 870 875 880

Leu Glu Gln Leu Leu Thr Asp Pro His Ser Leu Arg Thr Ile Ala Asn
885 890 895

Phe Asp Pro Arg Val Thr Leu Arg Leu Pro Ser Leu Ser Gly Ser Asp
900 905 910

Gly Ile Pro Tyr Arg Ser Val Ser Glu Trp Leu Glu Ser Ile Arg Met
915 920 925

Lys Arg Tyr Ile Leu His Phe Arg Ser Ala Gly Leu Asp Thr Met Glu
930 935 940

Cys Val Leu Glu Leu Thr Ala Glu Asp Leu Thr Gln Met Gly Ile Thr
945 950 955 960

Leu Pro Gly His Gln Lys Arg Ile Leu Cys Ser Ile Gln Gly Phe Lys
965 970 975

Asp

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GTAGGCATGC AAGGAGAC(A/C) TT(C/T)AACC 27

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGATGATCA T(C/G)AC(A/G/T)GA(A/G)TA (C/T)ATGG 25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTAGGAATTC CA(C/G/T)ACATC(A/G)C T(A/G)GC 24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCA(T/A)A(A/G)CTCC A(C/T)ACATC(A/G)CT 20

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asp Leu Ala Ala Arg Asn
5

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Ile Arg Trp Thr Ala Pro
5

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Glu Glu Val Thr Leu Met Asp Thr
                5
```

CLAIMS:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a receptor tyrosine kinase (RTK) or a derivative, homologue or chemical analogue thereof, said RTK having the following characteristics:
(i) belongs to the Eph subfamily of RTKs as determined by conserved cysteine residues and fibronectin type III repeats;
(ii) comprises protein tyrosine kinase catalytic domain motifs; and
(iii) comprises an amino acid sequence substantially as set forth in SEQ ID NO: 2 or having at least about 79% similarity thereto.

2. An isolated nucleic acid molecule according to claim 1 wherein the RTK is of mammalian origin.

3. An isolated nucleic acid molecule according to claim 2 wherein the RTK is of human or murine origin.

4. An isolated nucleic acid molecule according to claim 1 comprising a nucleotide sequence substantially as set forth in SEQ ID NO: 1 or having at least about 82% similarity thereto or is capable of hybridizing to the sequence set forth in SEQ ID NO: 1 or a complementary form thereof under low stringency conditions.

5. An isolated nucleic acid molecule according to claim 1 or 2 or 3 or 4 contained in a vector.

6. An isolated nucleic acid molecule according to claim 5 wherein the vector is an expression vector.

7. An isolated nucleic acid molecule according to claim 1 or 4 encoding a soluble form of said RTK.

8. A recombinant polypeptide or a derivative, homologue or chemical analogue thereof having the following characteristics:

(i) is an RTK belonging to the Eph subfamily of RTKs as determined by cysteine residues and fibronectin type II repeats;

(ii) comprises protein tyrosine kinase catalytic domain motifs; and

(iii) comprises an amino acid sequence substantially as set forth in SEQ ID NO:2 or having at least about 79% similarity thereto.

9. A recombinant polypeptide according to claim 8 wherein said RTK is of mammalian origin.

10. A recombinant polypeptide according to claim 9 wherein the RTK is of human or murine origin.

11. A recombinant polypeptide according to claim 8 encoded by a nucleotide sequence substantially as set forth in SEQ ID NO: 1 or having at least about 82% similarity thereto or a nucleotide sequence capable of hybridizing to the sequence set forth in SEQ ID NO: 1 or a complementary form thereof under low stringency conditions.

12. A recombinant polypeptide according to any one of claims 8 to 11 in soluble form.

13. A secreted recombinant polypeptide comprising conserved cysteine residues and fibronectin type III repeats and an extracellular portion of the amino acid sequence set forth in SEQ ID NO:2 or having at least about 79% similarity thereto.

14. A pharmaceutical composition comprising a recombinant polypeptide according to any one of claims 8 to 13 and one or more pharmaceutically acceptable carriers and/or diluents.

15. A method for modulating Esk-ligand interaction in an animal, said method comprising administering to said animal a modulating effective amount of an agonist or antagonist of Esk-

ligand interaction.

16. A method according to claim 15 wherein the Esk has the following characteristics:

(i) is an RTK belonging to the Eph subfamily of RTKs as determined by cysteine residues and fibronectin type II repeats;

(ii) comprises protein tyrosine kinase catalytic domain motifs; and

(iii) comprises an amino acid sequence substantially as set forth in SEQ ID NO: 2 or having at least about 79% similarity thereto.

17. An antagonist or agonist of an Esk, said Esk having the following characteristics:

(i) is an RTK belonging to the Eph subfamily of RTKs as determined by cysteine residues and fibronectin type II repeats;

(ii) comprises protein tyrosine kinase catalytic domain motifs; and

(iii) comprises an amino acid sequence substantially as set forth in SEQ ID NO: 2 or having at least about 79% similarity thereto.

18. An antagonist according to claim 17 wherein said antagonist is an antibody to said Esk.

19. An antibody to the recombinant polypeptide of claim 8.

20. An antibody to the secreted recombinant polypeptide of claim 13.

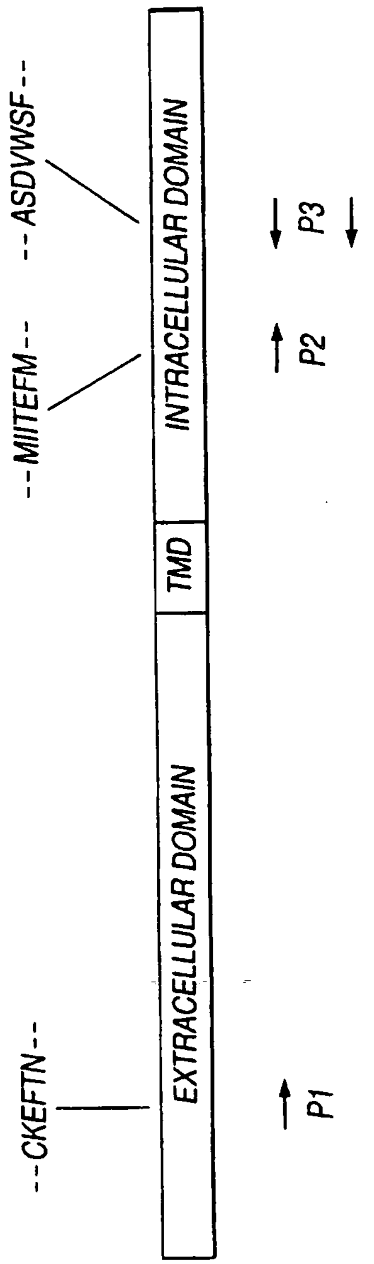
--CKEFTN--
--MIITEFM--
--ASDVWSF--
EXTRACELLULAR DOMAIN
TMD
INTRACELLULAR DOMAIN
P1
P2
P3
P4

Fig. 1

3/30	4/30
5/30	6/30
7/30	8/30
9/30	10/30
11/30	12/30
13/30	14/30
15/30	16/30
17/30	18/30
19/30	20/30
21/30	22/30

Fig. 2

Tatggtgaac cgtgtgtgtc tgctgcatga

Gtgctgtagt ggatccctgg tggaaaggcg

Aacatatgtc cccaaagagt ttaaagctga

Acttccagag aaggagaagc agattaagaa

Caagcccaag gctacagcgg agcaactgaa

Ggatacatgt tgcaaggctg ctgggaattc

ATG GAG CGG CGC TGG CCC CTG GGG
Met Glu Arg Arg Trp Pro Leu Gly

TGC GCC CCG CTG CCC CCG GGG GCG
Cys Ala Pro Leu Pro Pro Gly Ala

GAC ACA AGC ACA GCA CAA GGA GAG
Asp Thr Ser Thr Ala Gln Gly Glu

Fig. 2 (1)

gaagacccca gtgagtgagc atgttaccaa								-295
gccatgcttc tctgctctga cagttgatga								-235
gaccttcacc ttccactctg atatctgcac								-175
acaaacggct cttgctgagc tggtgaagca								-115
gactgtcatg gatgactttg cacagttcct								-55
ctgggccagg tcccggccgg cgcc								-1
CTT	GCA	TTG	CTG	CTG	CTG	CTG	CTC	48
Leu	Ala	Leu	Leu	Leu	Leu	Leu	Leu	16
CGC	GCC	GAG	GAA	GTC	ACT	CTA	ATG	96
Arg	Ala	Glu	Glu	Val	Thr	Leu	Met	32
CTG	GGC	TGG	CTT	CTG	GAT	CCC	CCA	144
Leu	Gly	Trp	Leu	Leu	Asp	Pro	Pro	48

Fig. 2 (II)

GAG	ACT	GGG	TGG	AGT	GAG	GTG	CAA
Glu	Thr	Gly	Trp	Ser	Glu	Val	Gln
TAC	ATG	TAC	CAA	GAC	TGC	CCA	ATA
Tyr	Met	Tyr	Gln	Asp	Cys	Pro	Ile
TGG	CTT	CGC	TCC	AAT	TGG	ATC	TAC
Trp	Leu	Arg	Ser	Asn	Trp	Ile	Tyr
TAC	GTG	GAG	CTG	CAG	TTC	ACC	GTG
Tyr	Val	Glu	Leu	Gln	Phe	Thr	Val
GGA	GCT	GGG	CCT	CTG	GGA	TGC	AAA
Gly	Ala	Gly	Pro	Leu	Gly	Cys	Lys
GAG	AGT	GAC	CAG	GAT	GTG	GGC	ATT
Glu	Ser	Asp	Gln	Asp	Val	Gly	Ile
AAG	GTA	ACA	ACT	GTG	GCA	GCA	GAC
Lys	Val	Thr	Thr	Val	Ala	Ala	Asp

Fig. 2 (III)

CAA	ATG	CTA	AAC	GGG	ACA	CCC	CTG	192
Gln	Met	Leu	Asn	Gly	Thr	Pro	Leu	64
CAG	GAA	GGT	GGG	GAC	ACT	GAC	CAC	240
Gln	Glu	Gly	Gly	Asp	Thr	Asp	His	80
CGC	GGA	GAG	GAA	GCT	TCA	CGC	ATC	288
Arg	Gly	Glu	Glu	Ala	Ser	Arg	Ile	80
CGG	GAC	TGT	AAG	AGT	TTC	CCA	GGG	336
Arg	Asp	Cys	Lys	Ser	Phe	Pro	Gly	112
GAG	ACC	TTC	AAC	CTT	TTC	TAC	ATG	384
Glu	Thr	Phe	Asn	Leu	Phe	Tyr	Met	128
CAG	CTC	CGA	CGA	CCT	TTG	TTC	CAA	432
Gln	Leu	Arg	Arg	Pro	Leu	Phe	Gln	144
CAG	AGC	TTC	ACC	ATC	AGA	GAC	CTG	480
Gln	Ser	Phe	Thr	Ile	Arg	Asp	Leu	160

Fig. 2 (IV)

GCA	TCT	GAC	TCT	GTA	AAG	CTG	AAT
Ala	Ser	Asp	Ser	Val	Lys	Leu	Asn
CTC	ACC	CGC	CGT	GGC	CTC	TAC	TTA
Leu	Thr	Arg	Arg	Gly	Leu	Tyr	Leu
GTG	GCG	CTA	GTG	TCT	GTA	AGG	GTG
Val	Ala	Leu	Val	Ser	Val	Arg	Val
GTG	CAT	GGC	TTG	GCC	CAC	TTC	CCT
Val	His	Gly	Leu	Ala	His	Phe	Pro
TTG	GTT	GAA	GTA	GCT	GGA	ACG	TGC
Leu	Val	Glu	Val	Ala	Gly	Thr	Cys
GGG	TCC	TCA	GGT	ACA	CCA	CGA	ATG
Gly	Ser	Ser	Gly	Thr	Pro	Arg	Met
CTG	GTG	CCT	GTG	GGT	CAG	TGC	CAG
Leu	Val	Pro	Val	Gly	Gln	Cys	Gln

Fig. 2 (V)

GTA	GAA	CGC	TGC	TCG	TTG	GGC	CAC	528
Val	Glu	Arg	Cys	Ser	Leu	Gly	His	176
GCT	TTC	CAC	AAC	CCG	GGT	TCC	TGT	576
Ala	Phe	His	Asn	Pro	Gly	Ser	Cys	192
TTC	TAC	CAG	CGC	TGT	GCC	GAG	ACC	624
Phe	Tyr	Gln	Arg	Cys	Ala	Glu	Thr	208
GAC	ACT	CTC	CCT	GGA	CCT	GGA	GGG	672
Asp	Thr	Leu	Pro	Gly	Pro	Gly	Gly	224
CTC	TCC	CAT	GCA	CAG	ATC	AGC	TTG	720
Leu	Ser	His	Ala	Gln	Ile	Ser	Leu	240
CAC	TGC	AGC	CCT	GAT	GGC	GAG	TGG	768
His	Cys	Ser	Pro	Asp	Gly	Glu	Trp	256
TGC	GAG	CCT	GGC	TAT	GAA	GAA	AGC	816
Cys	Glu	Pro	Gly	Tyr	Glu	Glu	Ser	272

Fig. 2 (VI)

AGT GGA AAT GTG GGA TGC ACT GCC
Ser Gly Asn Val Gly Cys Thr Ala

GAC ATG AAT ACA CTC CGT TGT CTC
Asp Met Asn Thr Leu Arg Cys Leu

GAG TCT GAG GGG TCT ACC ATC TGT
Glu Ser Glu Gly Ser Thr Ile Cys

GCC CCT GGG GAG GGT CCC CAG GTA
Ala Pro Gly Glu Gly Pro Gln Val

CCC CAA AAT CTG AGC TTC TCC ACA
Pro/Gln/Asn/Leu/Ser/Phe/Ser/Thr/

TGG GAG CCC CCC AGA GAT ACA GGG
Trp/Glu/Pro/Pro/Arg/Asp/Thr/Gly/

GTG GAG TGC TTG CAG TGT CGG GGC
Val/Glu/Cys/Leu/Gln/Cys/Arg/Gly/

Fig. 2 (VII)

TGT	CCT	ACT	GGT	TTC	TAT	CGA	GTG	864
Cys	Pro	Thr	Gly	Phe	Tyr	Arg	Val	288
AAG	TGC	CCC	CAA	CAT	AGC	ATA	GCA	912
Lys	Cys	Pro	Gln	His	Ser	Ile	Ala	304
ACC	TGT	GAG	AAT	GGA	CAT	TAT	CGA	960
Thr	Cys	Glu	Asn	Gly	His	Tyr	Arg	320
GCA	TGC	ACA	CGT	CCC	CCA	TCG	GCT	1008
Ala	Cys	Thr/	Arg/	Pro/	Pro/	Ser/	Ala	336
TCA	GGG	ACT	CAA	CTC	TCC	CTG	CGC	1056
Ser/	Gly/	Thr/	Gln/	Leu/	Ser/	Leu/	Arg	352
GGA	CGC	CAT	GAT	ATC	AGA	TAC	AGC	1104
Gly/	Arg/	His/	Asp/	Ile/	Arg/	Tyr/	Ser	368
ATT	GCA	CAG	GAT	GGG	GGT	CCC	TGC	1152
Ile/	Ala/	Gln/	Asp/	Gly/	Gly/	Pro/	Cys	384

Fig. 2 (VIII)

CAA	CCC	TGT	GGA	AAA	GGT	GTG	CAC
Gln/	Pro/	Cys/	Gly/	Lys/	Gly/	Val/	His/
ACC	ACA	TCT	ACC	GTG	CAA	GTG	CAA
Thr/	Thr/	Ser/	Thr/	Val/	Gln/	Val/	Gln/
ACA	TTT	ACC	GTC	AAA	TCC	CAA	AAC
Thr/	Phe/	Thr/	Val/	Lys/	Ser/	Gln/	Asn/
AGC	CCT	AGC	AGC	GCC	TCC	CTC	AGT
Ser/	Pro/	Ser/	Ser/	Ala/	Ser/	Leu/	Ser/
CTC	TCT	GGC	CTG	TCA	CTG	AAG	CTG
Leu/	Ser/	Gly/	Leu/	Ser/	Leu/	Lys/	Leu/
GAG	CTG	ACT	TGG	GCA	GGG	TCC	CGA
Glu/	Leu/	Thr/	Trp/	Ala/	Gly/	Ser/	Arg/
AGC	TAT	GAG	CTG	CAC	GTG	CTG	AAT
Ser/	Tyr/	Glu/	Leu/	His/	Val/	Leu/	Asn/

Fig. 2 (IX)

TTT TCC CCG GCT GCT TCC GGG CTC	1200
Phe/Ser/Pro/Ala/Ala/Ser/Gly/Leu	400
GGC CTC GAG CCT TAC GCC AAC TAC	1248
Gly/Leu/Glu/Pro/Tyr/Ala/Asn/Tyr	416
AGA GTG TCA GGA CTG GAC AGT TCC	1296
Arg/Val/Ser/Gly/Leu/Asp/Ser/Ser	432
ATC AAC ATG GGG CAC GCA GAG TCA	1344
Ile Asn Met Gly His Ala Glu Ser	448
GTG AAG AAA GAA CCG AGG CAG CTG	1392
Val/Lys/Lys/Glu/Pro/Arg/Gln/Leu	464
CCC CGA AAT CCT GGA GGG AAT CTG	1440
Pro/Arg/Asn/Pro/Gly/Gly/Asn/Leu	480
CAG GAC GAA GAA TGG CAC CAG ATG	1488
Gln/Asp/Glu/Glu/Trp/His/Gln/Met	496

Fig. 2 (X)

GTG	TTG	GAA	CCC	AGG	GTC	TTG	CTG
Val/	Leu/	Glu/	Pro/	Arg/	Val/	Leu/	Leu/
TAC	ATT	GTC	AGA	GTG	CGA	ACA	CTG
Tyr/	Ile/	Val/	Arg/	Val/	Arg/	Thr/	Leu/
TCC	CCT	GAC	CAT	GAG	TTT	CGG	ACA
Ser/	Pro/	Asp/	His/	Glu/	Phe/	Arg/	Thr
ACC	GGA	GGA	GAG	ATT	GTG	GCC	GTC
Thr	Gly	Gly	Glu	Ile/	Val/	Ala/	Val/
GCT	CTG	CTG	ATC	GGG	ATT	TAT	GTC
Ala/	Leu/	Leu/	Ile/	Gly/	Ile/	Tyr/	Val/
CAG	AGA	CAG	CAG	AGG	CAG	CGT	GAA
Gln	Arg	Gln	Gln	Arg	Gln	Arg	Glu
GAC	AAG	CTG	TGG	CTA	AAA	CCC	TAT
Asp	Lys	Leu	Trp	Leu	Lys	Pro	Tyr

Fig. 2 (XI)

ACA AAA CTT CAG CCA GAT ACC ACA	1536
Thr/Lys/Leu/Gln/Pro/Asp/Thr/Thr	512
ACC CCA CTG GGG CCT GGC CCT TTC	1584
Thr/ Pro/ Leu/ Gly/ Pro/ Gly/ Pro/ Phe	528
AGC CCA CCA GTT TCC AGA AGC CTG	1632
Ser Pro Pro Val Ser Arg Ser Leu	544
ATC TTT GGA TTG CTG CTT GGA ATA	1680
Ile/Phe/Gly/Leu/Leu/Leu/Gly/Ile	560
TTC CGT TCA AGG AGA GGC CAG AGA	1728
Phe Arg Ser Arg Arg Gly Gln Arg	576
CGC ACC ACC AAT GTC GAT CGA GAG	1776
Arg Thr Thr Asn Val Asp Arg Glu	592
GTG GAC CTC CAG GCC TAT GAG GAC	1824
Val Asp Leu Gln Ala Tyr Glu Asp	608

Fig. 2 (XII)

CCT	GCA	CAG	GGA	GCC	TTA	GAC	TTT
Pro	Ala	Gln	Gly	Ala	Leu	Asp	Phe
CTG	ATT	GTG	GAC	ACT	GTC	ATA	GGA
Leu	Ile	Val	Asp	Thr	Val	Ile	Gly ▲
CGG	GGA	GCC	CTG	AGA	CTC	CCC	AGC
Arg	Gly	Ala	Leu	Arg	Leu	Pro	Ser
AAG	ACC	TTG	AAA	GAT	ACA	TCC	CCA
Lys •	Thr	Leu	Lys	Asp	Thr	Ser	Pro
CGA	GAG	GCA	ACT	ATC	ATG	GGC	CAG
Arg	Glu	Ala	Thr	Ile	Met	Gly	Gln
CTA	GAA	GGT	GTC	ATC	ACA	AAA	AGA
Leu	Glu	Gly	Val	Ile	Thr	Lys	Arg
TTT	ATG	GAA	AAT	GGA	GCC	CTG	GAT
Phe	Met	Glu	Asn	Gly	Ala	Leu	Asp

Fig. 2 (XIII)

GCC	CAG	GAA	CTG	GAC	CCA	GCC	TGG	1872
Ala	Gln	Glu	Leu	Asp	Pro	Ala	Trp	624
GAA	GGG	GAG	TTT	GGT	GAA	GTG	TAT	1920
Glu	Gly ▲	Glu	Phe	Gly ▲	Glu	Val	Tyr	640
CAA	GAT	TGC	AAG	ACT	GTG	GCC	ATT	1968
Gln	Asp	Cys	Lys	Thr	Val	Ala	Ile	656
GAT	GGC	TAC	TGG	TGG	AAT	TTC	CTT	2016
Asp	Gly	Tyr	Trp	Trp	Asn	Phe	Leu	672
TTC	AAC	CAC	CCA	CAC	ATT	CTA	CGC	2064
Phe	Asn	His	Pro	His	Ile	Leu	Arg	688
AAG	CCC	ATC	ATG	ATC	ATC	ACA	GAA	2112
Lys	Pro	Ile	Met	Ile	Ile	Thr	Glu	704
GCC	TTT	CTG	AAG	GAA	CGG	GAG	GAC	2160
Ala	Phe	Leu	Lys	Glu	Arg	Glu	Asp	720

Fig. 2 (XIV)

CAA	CTA	GCT	CCT	GGT	CAG	CTA	GTG
Gln	Leu	Ala	Pro	Gly	Gln	Leu	Val
GGC	ATG	AAC	TGC	CTC	AGT	GGC	CAC
Gly	Met	Asn	Cys	Leu	Ser	Gly	His
GCC	AGG	AAC	ATC	TTG	GTG	AAT	CAG
Ala	Arg	Asn	Ile	Leu	Val	Asn	Gln
TTT	GGC	TTG	ACC	CGC	CTC	CTG	GAT
Phe	Gly	Leu	Thr	Arg	Leu	Leu	Asp
CAG	GGA	GGA	AAG	ATC	CCC	ATC	CGA
Gln	Gly	Gly	Lys	Ile	Pro	Ile	Arg
CAT	CGG	ATC	TTC	ACC	ACA	GCC	AGT
His	Arg	Ile	Phe	Thr	Thr	Ala	Ser
ACG	TGG	GAG	GTG	TTG	AGT	TTT	GGC
Thr	Trp	Glu	Val	Leu	Ser	Phe	Gly

Fig. 2 (XV)

GCT	ATG	CTA	CTG	GGC	ATA	GCA	TCA	2208
Ala	Met	Leu	Leu	Gly	Ile	Ala	Ser	736
AAT	TAT	GTC	CAT	AGA	GAC	CTG	GCT	2256
Asn	Tyr	Val	His	Arg	Asp	Leu	Ala	752
AAC	CTG	TGC	TGC	AAG	GTG	TCT	GAC	2304
Asn	Leu	Cys	Cys	Lys	Val	Ser	Asp	768
GAC	TTT	GAC	GGC	ACC	TAT	GAA	ACC	2352
Asp	Phe	Asp	Gly	Thr	Tyr	Glu	Thr	784
TGG	ACA	GCC	CCA	GAA	GCT	ATT	GCC	2400
Trp	Thr	Ala	Pro	Glu	Ala	Ile	Ala	800
GAT	GTG	TGG	AGC	TTT	GGG	ATT	GTA	2448
Asp	Val	Trp	Ser	Phe	Gly	Ile	Val	816
GAC	AAA	CCC	TAT	GGG	GAG	ATG	AGC	2496
Asp	Lys	Pro	Tyr	Gly	Glu	Met	Ser	832

Fig. 2 (XVI)

AAC	CAA	GAG	GTA	ATG	AAA	AGC	ATT
Asn	Gln	Glu	Val	Met	Lys	Ser	Ile
CCT	GTG	GAC	TGT	CCT	GCC	CCT	CTC
Pro	Val	Asp	Cys	Pro	Ala	Pro	Leu
GCT	TAC	GAT	CGT	GCC	CGT	CGA	CCC
Ala	Tyr	Asp	Arg	Ala	Arg	Arg	Pro
CTG	GAA	CAG	TTG	CTT	ACT	GAC	CCC
Leu	Glu	Gln	Leu	Leu	Thr	Asp	Pro
TTT	GAC	CCT	AGG	GTG	ACC	TTA	CGC
Phe	Asp	Pro	Arg	Val	Thr	Leu	Arg
GGG	ATC	CCT	TAT	CGA	AGT	GTC	TCT
Gly	Ile	Pro	Tyr	Arg	Ser	Val	Ser
AAG	CGC	TAC	ATC	CTG	CAC	TTC	CGT
Lys	Arg	Tyr	Ile	Leu	His	Phe	Arg

Fig. 2 (XVII)

GAA	GAT	GGG	TAC	CGG	TTG	CCC	CCT	2544
Glu	Asp	Gly	Tyr	Arg	Leu	Pro	Pro	848
TAT	GAA	CTC	ATG	AAG	AAC	TGC	TGG	2592
Tyr	Glu	Leu	Met	Lys	Asn	Cys	Trp	864
CAC	TTC	CTC	CAG	CTG	CAG	GCA	CAT	2640
His	Phe	Leu	Gln	Leu	Gln	Ala	His	880
CAT	TCC	CTA	AGG	ACA	ATT	GCC	AAC	2688
His	Ser	Leu	Arg	Thr	Ile	Ala	Asn	896
CTG	CCC	AGC	CTG	AGT	GGC	TCT	GAT	2736
Leu	Pro	Ser	Leu	Ser	Gly	Ser	Asp	912
GAG	TGG	CTT	GAA	TCC	ATA	CGC	ATG	2784
Glu	Trp	Leu	Glu	Ser	Ile	Arg	Met	928
TCG	GCT	GGG	CTG	GAC	ACC	ATG	GAG	2832
Ser	Ala	Gly	Leu	Asp	Thr	Met	Glu	944

Fig. 2 (XVIII)

TGT GTG CTG GAG CTG ACG GCT GAG
Cys Val Leu Glu Leu Thr Ala Glu

TTG CCA GGG CAC CAG AAA CGA ATT
Leu Pro Gly His Gln Lys Arg Ile

GAC TGAGCATCCA CTGAAAAGAT GCTCC
Asp *

Aggacggggt acagtcaact ccctgggcct

Tgctgctcct gcccagtcaa tcagaactct

Agggggtgga tgggtacaag tgaaggggnc

natacactta catatgcatt atctattttt

Aaaaaaaaag gaattcctgc agcccggggg

Ggtggagctc cagcttttgt tcccttta

Fig. 2 (XIX)

```
GAC CTG ACG CAG ATG GGA ATA ACG   2880
Asp Leu Thr Gln Met Gly Ile Thr   960

CTC TGC AGT ATT CAA GGA TTT AAG   2928
Leu Cys Ser Ile Gln Gly Phe Lys   976

AGCCC TCTGCCTGCC TCCATTAGCA       2981
                                  977

ttcctcagcc tacgaaatgt aggctattgg  3041

gcctttgaac caaggagcct ttgtttataa  3101

tgtgggtggg ttctgggga gggtttaata   3161

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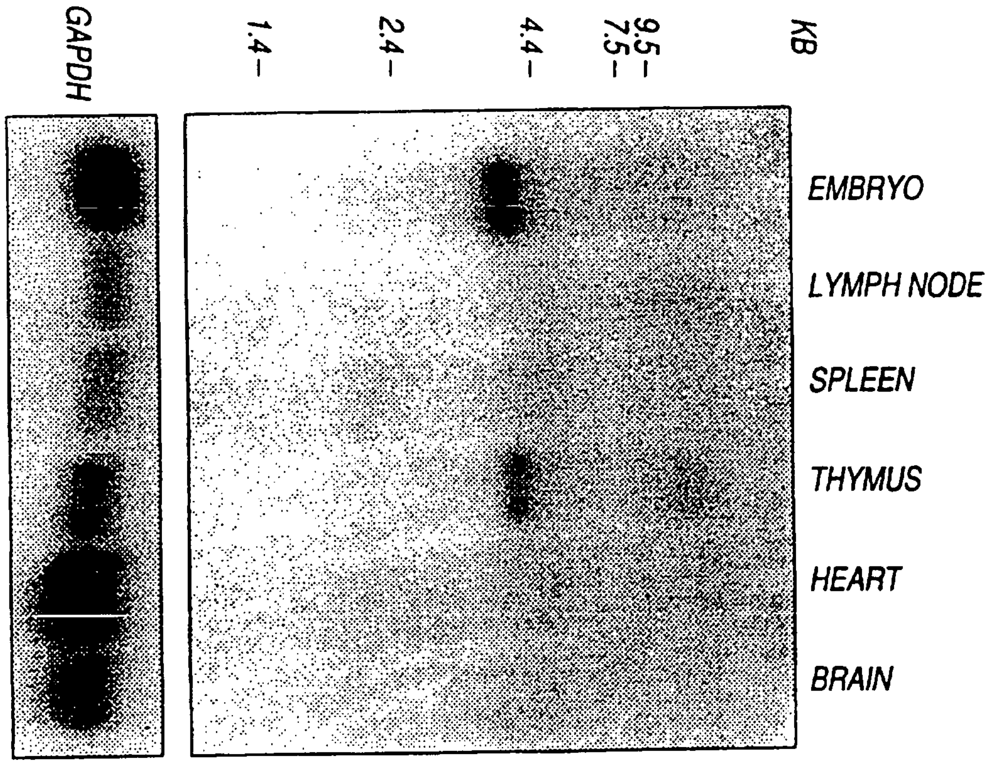
Fig. 2 (XX)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BRAIN

Fig. 3

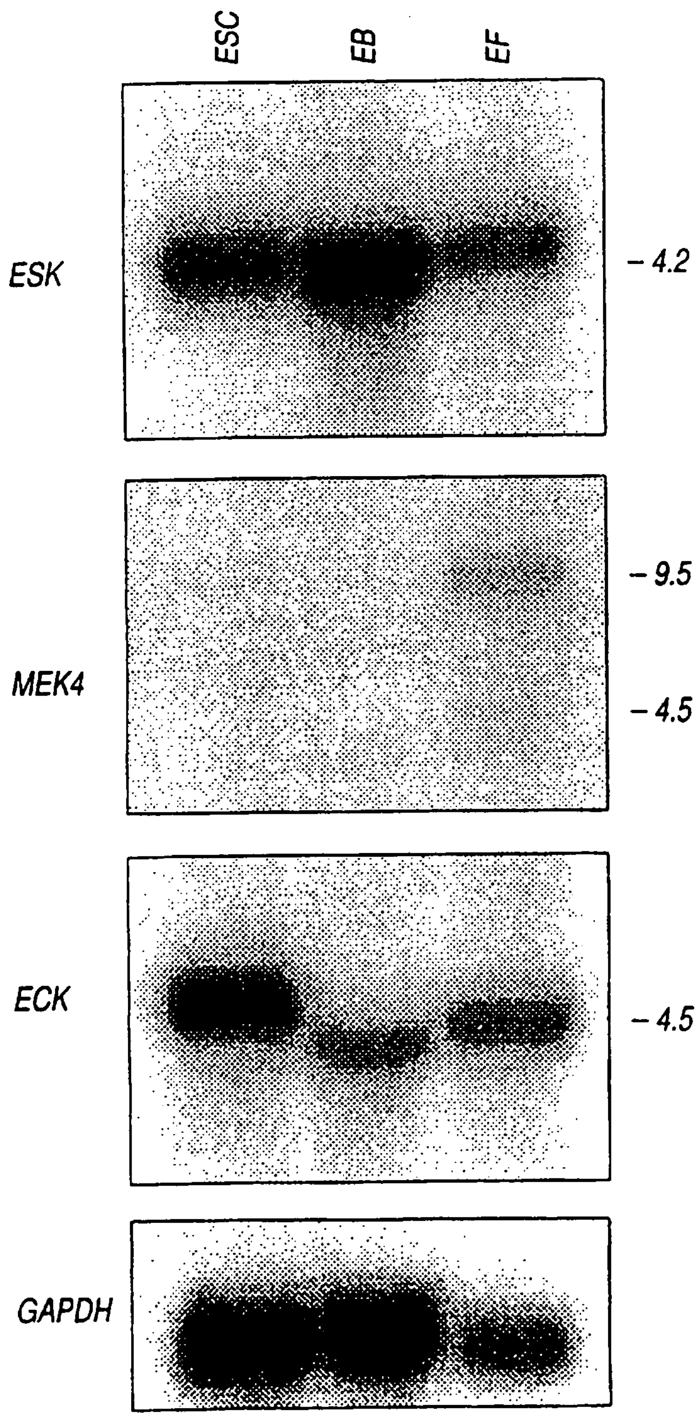

Fig. 4

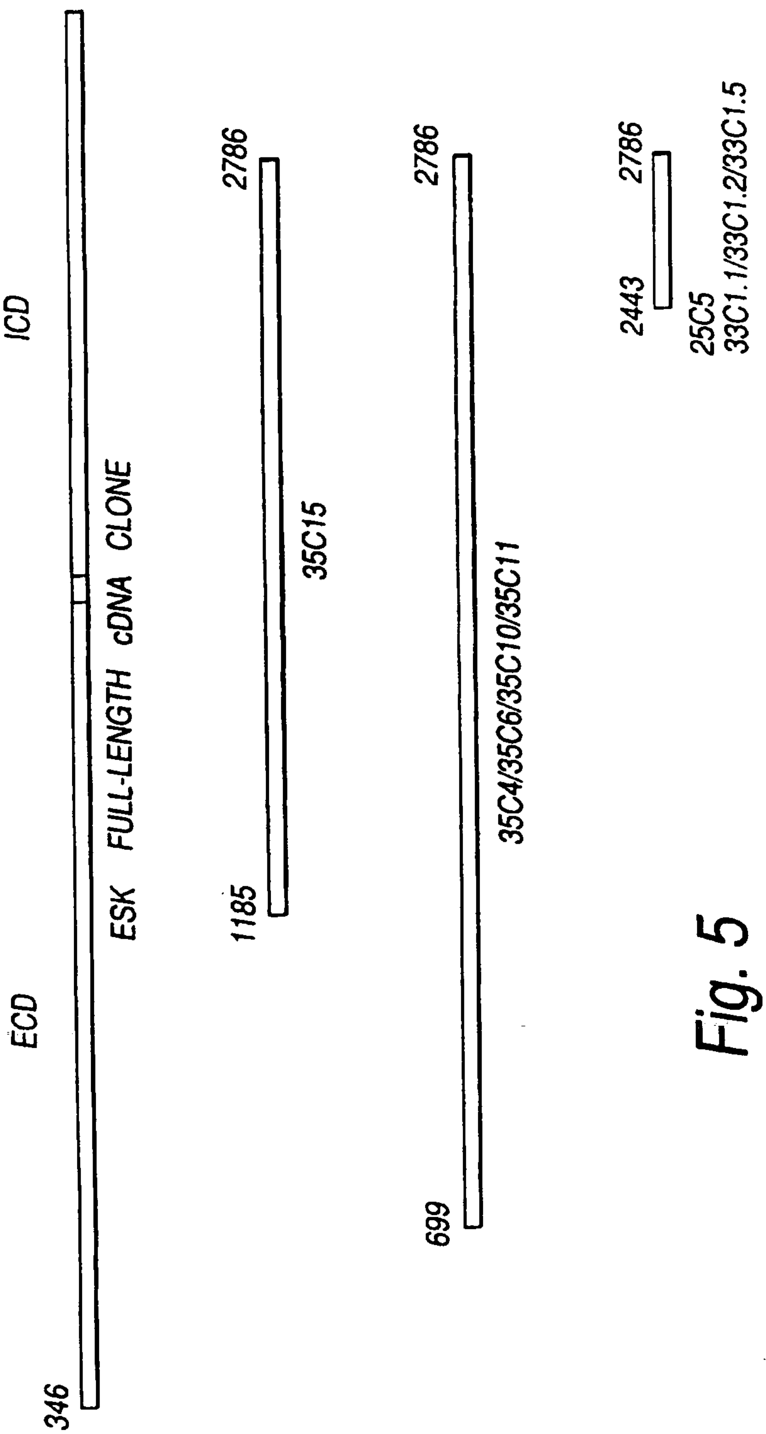
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Fig. 5

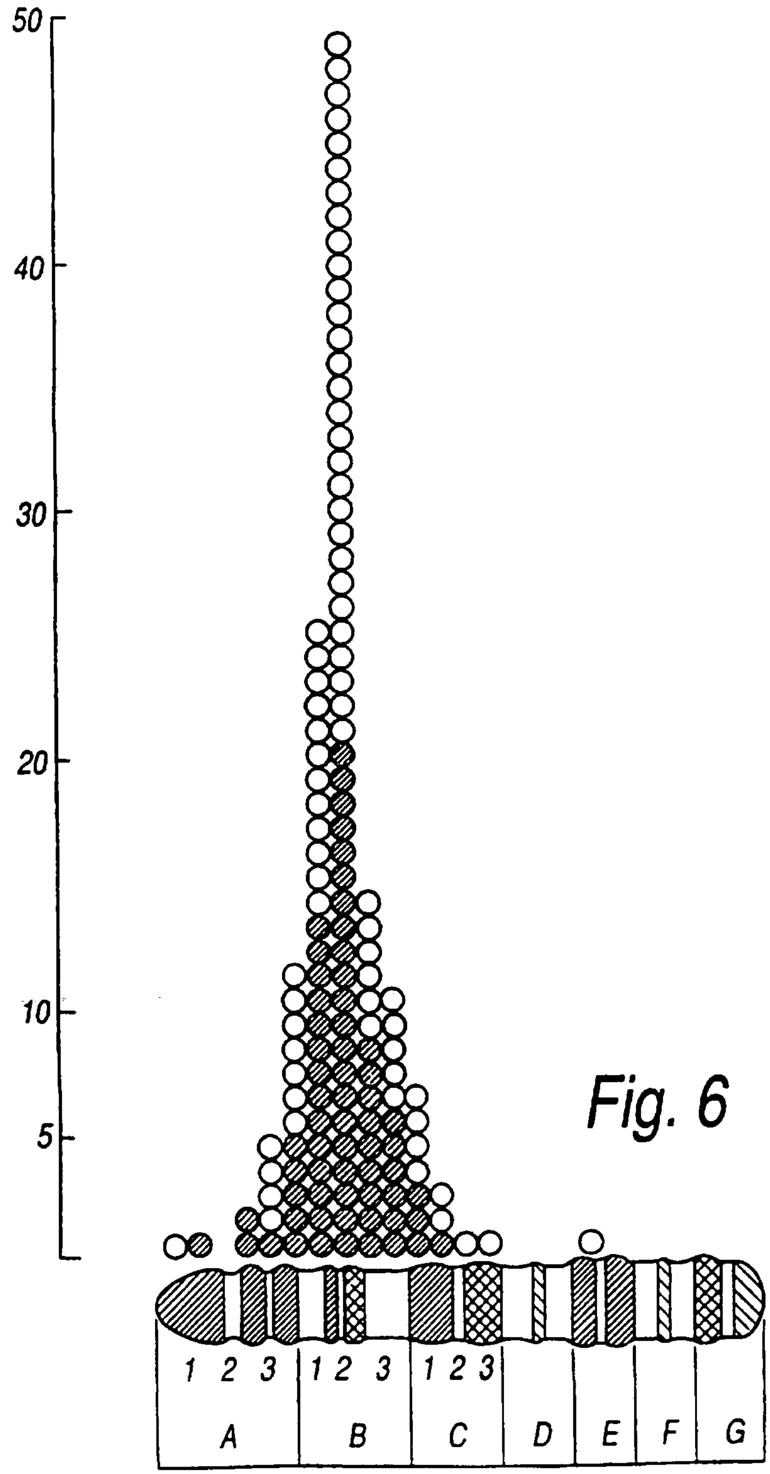
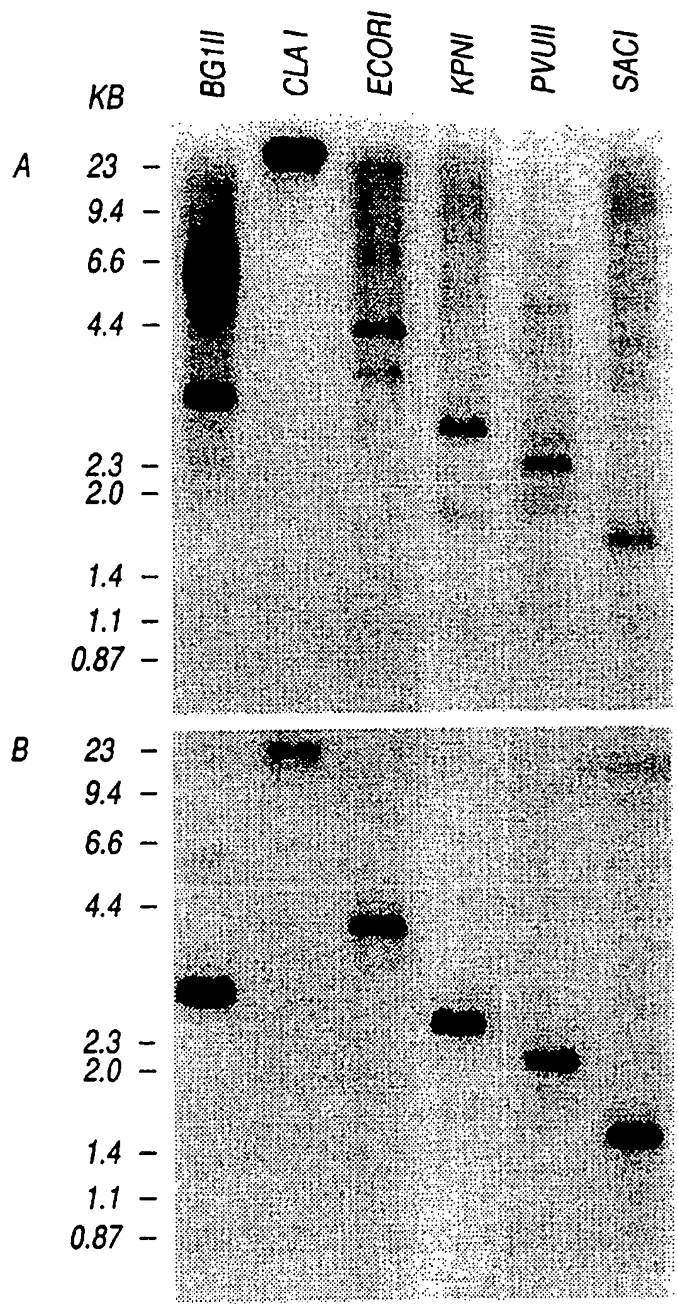


Fig. 6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1.1
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B
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4.4
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2.0
1.4
1.1
0.87

Fig. 7

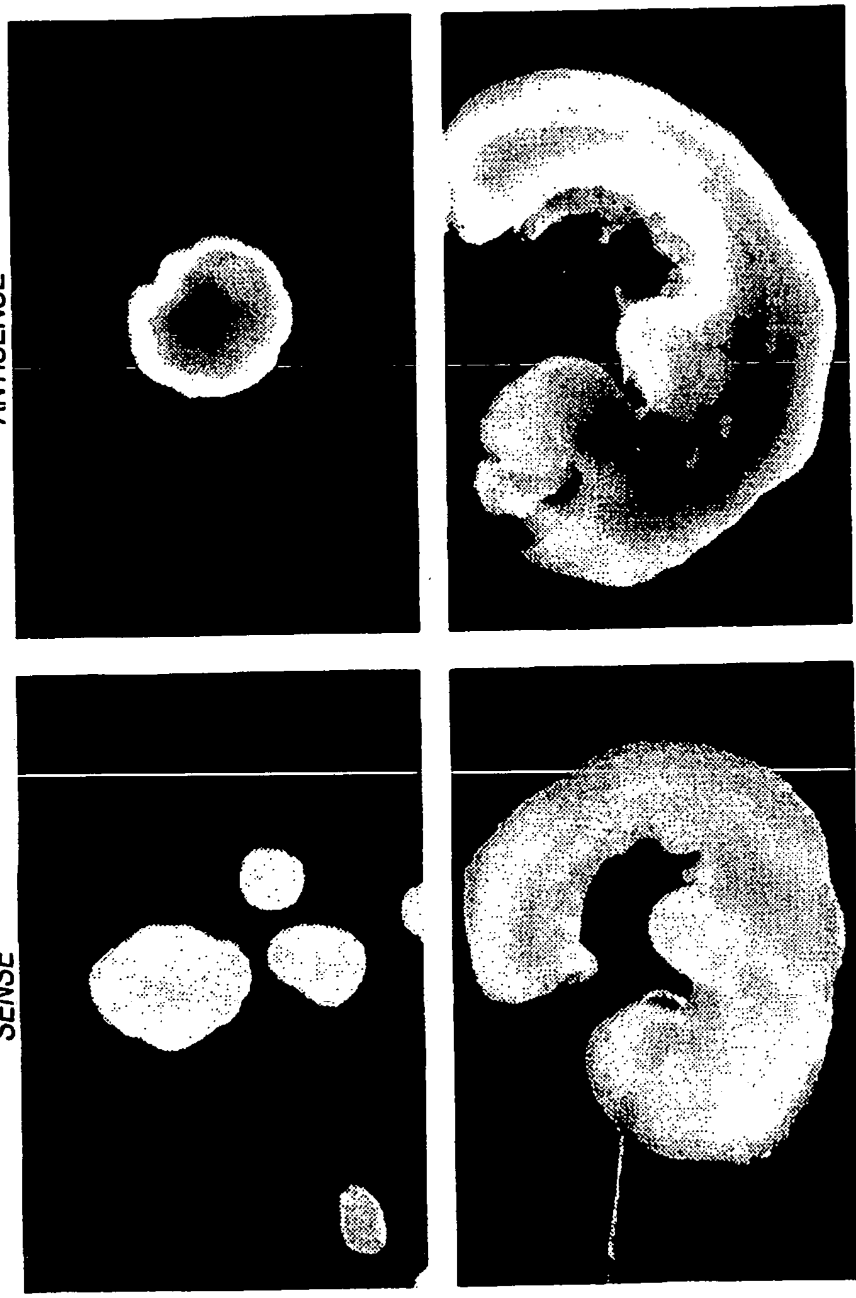


Fig. 8

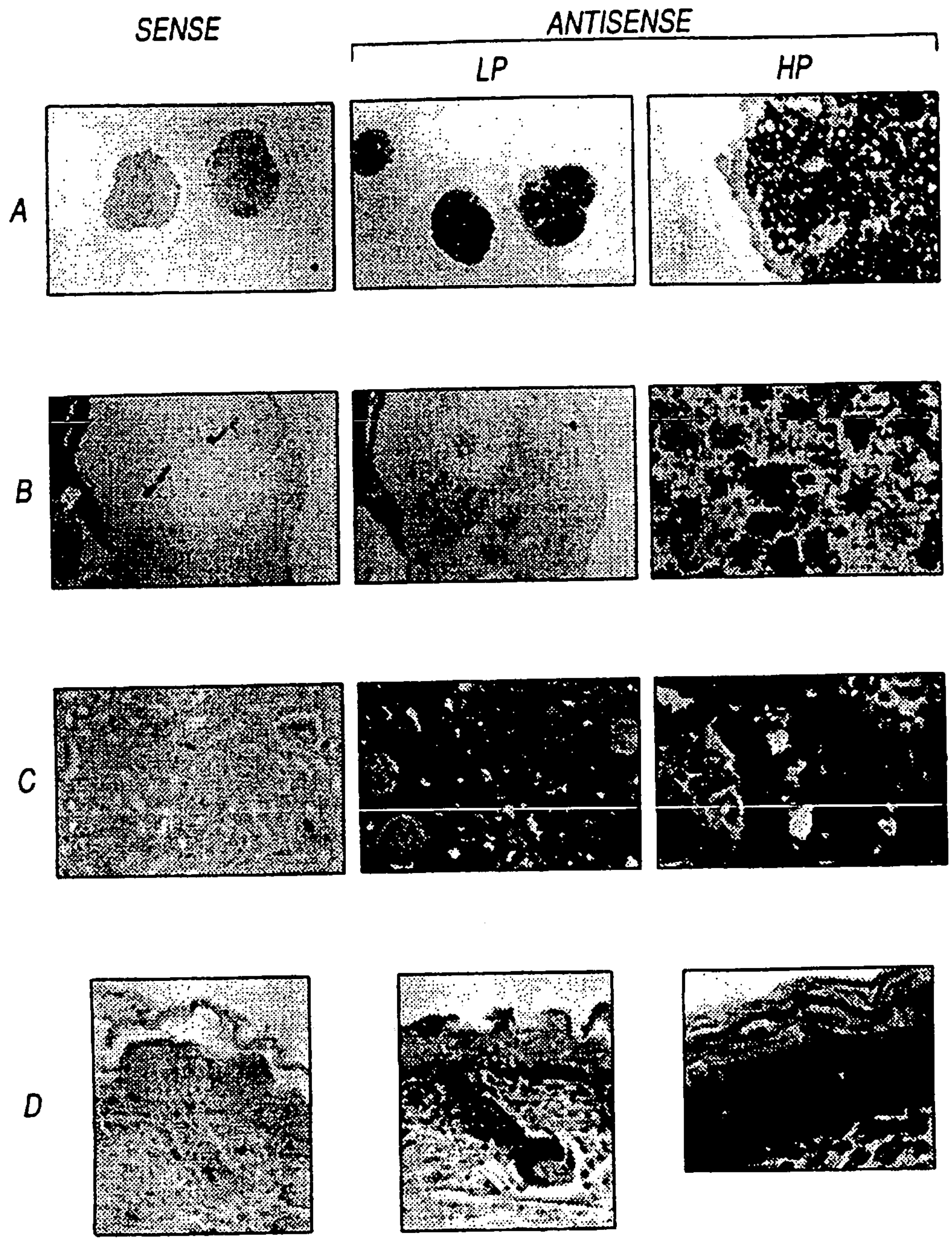
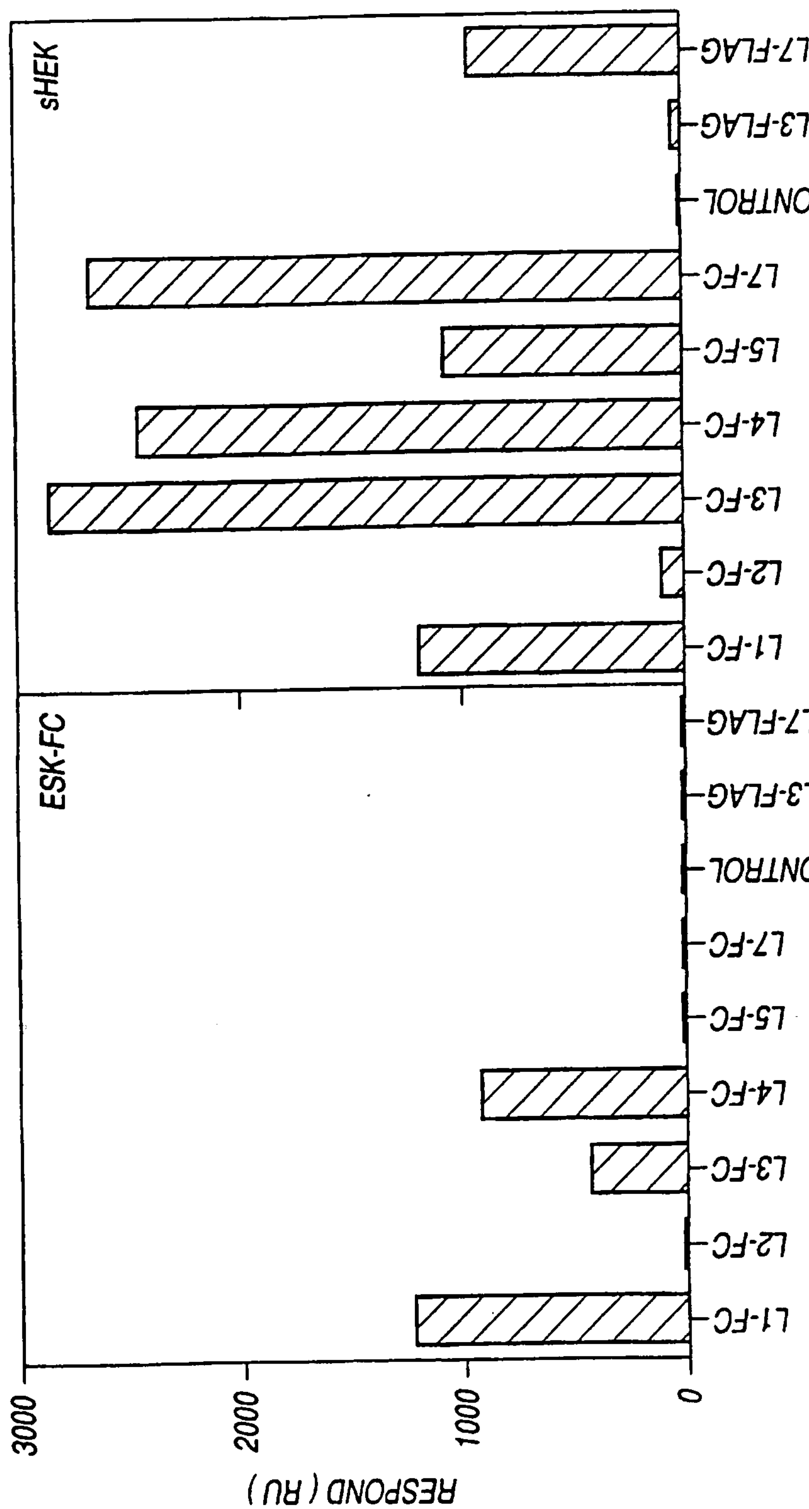


Fig. 9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L7-FC
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L4-FC
L3-FC
L2-FC
L1-FC
3000
2000
1000
0
RESPOND (RU)

Fig. 10

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00826

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl^{6}: C12N 15/54; C07K 14/71, 16/28; A61K 38/17, 39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Medline: Receptor protein-tyrosine kinase and EPH
Angis: full peptide sequence SEQ I.D. No.2

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See continuation

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	Proc. Natl. Acad. Sci. USA. Vol 93, pp 145-150, (January 1996), Lickliter et al. "Embryonic stem cells express multiple Eph-subfamily receptor tyrosime kinases" (see whole document)	1-20
X	Science, vol. 238 pp 1717-1720, (1987), Hirai et al. "A novel putative tyrosine kinase receptor encoded by the eph gene" (see whole document)	1-20
A	Embo Journal, vol 14(13) pp 3116-3126 (1995), Brambilla et al. "Membrane-bound LERK 2 ligand can signal through three different Eph-related receptor tyrosine kinases". (see whole document)	1-20

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex

* Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 February 1997	20 FEB 1997

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Form PCT/ISA/210 (second sheet) (July 1992) coprow

Box continuation of Box B

STN: Amino acid sequence
ECLQCRGI|AASGLTTSTVQVQ/SQSP

WPAT/USPM/JAPIO:
RTK OR RPTK OR RECEPTOR (W) PROTEIN (W) TYROSINE (W) KINASE# OR RECEPTOR (W) TYROSINE (W) KINASE#

CAS online:

L1 RTK OR RPTK OR RECEPTOR (W) PROTEIN (W) TYROSINE(W) KINASE# OR RECEPTOR (W) TYROSINE (W) KINASE#

L2 EPH

L3 L1 AND L2

L6 EPH (5A) RELATED

L7 L6 AND L3

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 96/00826

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Oncogene, vol. 10, pp 897-905 (1995), Fox et al., "cDNA cloning and tissue distribution of five human EPH-like receptor protein-tyrosine kinases" (see whole document)	1-20